Volume XXVII January, 1929 Number 1

SOIL SCIENCE

Editor-in-Chief
JACOB G. LIPMAN

Associate Editor
HERMINIE BROEDEL KITCHEN

Contents

PUBLISHED MONTHLY
THE WILLIAMS & WILKINS COMPANY
MT. ROYAL AND GUILFORD AVENUES
Baltimore, Maryland, U. S. A.

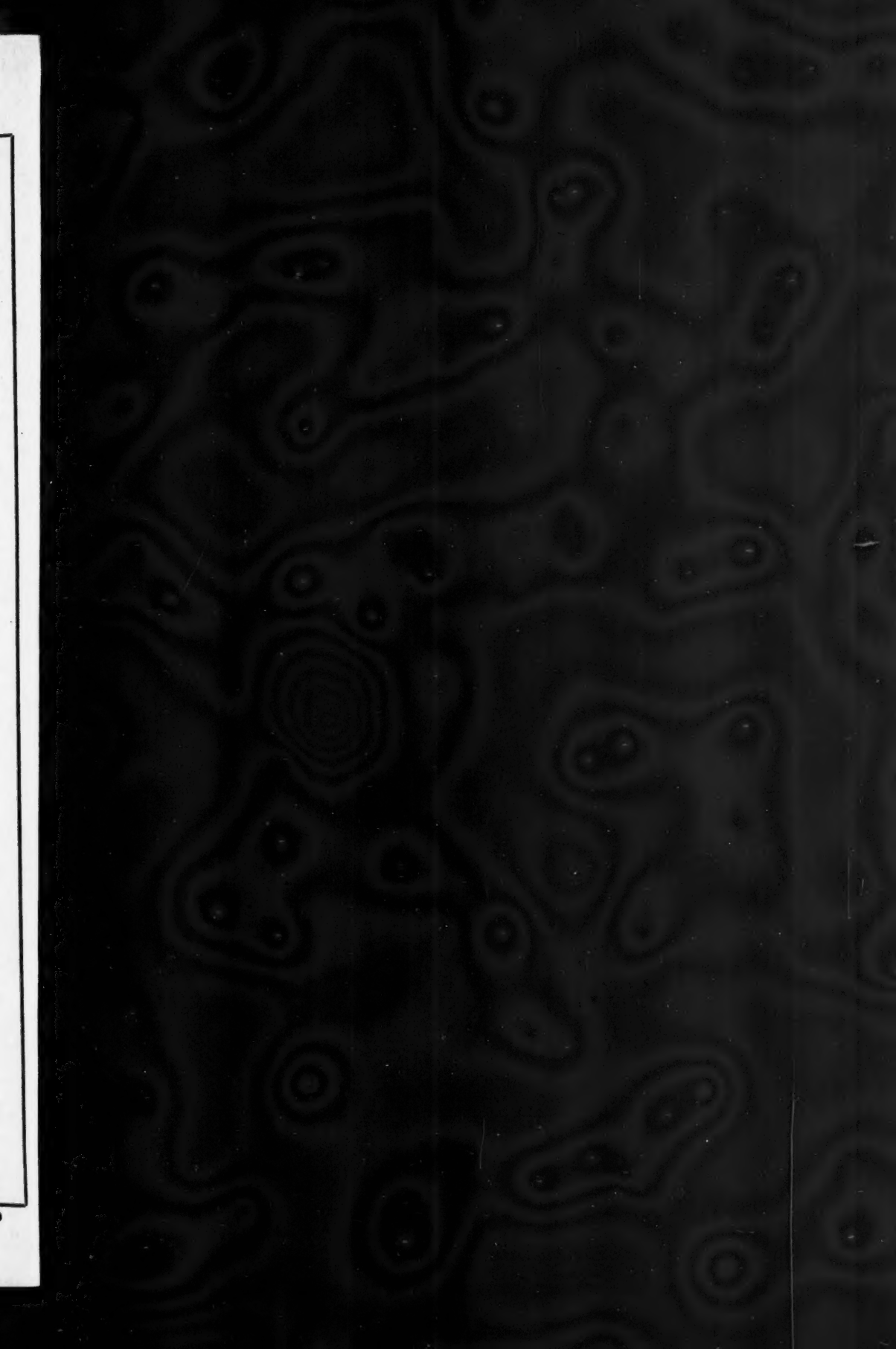
Made in United States of America

SOIL SCIENCE

FOUNDED BY
RUTGERS COLLEGE
NEW BRUNSWICK, N. J.

EDITORIAL BOARD

Entered as second-class matter May 12, 1919, at the post office at Baltimore, Maryland, under the act of March 3, 1879



SOIL SCIENCE

VOLUME XXVII

JANUARY - JUNE, 1929

RUTGERS UNIVERSITY
NEW BRUNSWICK, NEW JERSEY
U. S. A.

PUBLISHED BY
THE WILLIAMS & WILKINS COMPANY
BALTIMORE, MARYLAND

CONTENTS

ILLUSTRATIONS

PLATES

TEXT-FIGURES

A Study of the Nature of the Nitrogenous Compounds in Fungous Tissue and Their Decomposition in the Soil

The Influence of the Replaceable Bases on the Soil Solution Formation in Mineralized Soils

The Influence of Manganiferous Soils on the Accuracy of the Quinhydrone Electrode

The Measurement of "Suction Forces" in Soils

AN IMPROVED SOIL-SAMPLING TUBE

RELATION OF TEMPERATURE TO THE AMOUNT OF NITROGEN IN SOILS

A CRITICAL STUDY OF THE INFLUENCE OF SOIL TYPE ON THE CALCIUM AND MAGNESIUM CONTENT AND OTHER PHYSIOLOGICAL CHARACTERS OF THE ALFALFA PLANT

REPAIR OF SOIL FILTER TUBES

MICROBIOLOGICAL ACTIVITIES IN THE SOIL OF AN UPLAND BOG IN EASTERN NORTH CAROLINA

THE EFFECT OF MOISTURE CONTENT AND CROPPING ON EXCHANGEABLE CALCIUM AND MAGNESIUM, WITH PARTICULAR REFERENCE TO RICE SOIL

SOME INFLUENCE OF THE DEVELOPMENT OF HIGHER PLANTS UPON THE MICROÖRGANISMS IN THE SOIL: I. HISTORICAL AND INTRODUCTORY

SOME INFLUENCES OF THE DEVELOPMENT OF HIGHER PLANTS UPON THE MICROÖRGANISMS IN THE SOIL: II. INFLUENCE OF THE STAGE OF PLANT GROWTH UPON ABUNDANCE OF ORGANISMS

THE BOTANICAL COMPOSITION AND MORPHOLOGICAL FEATURES OF "HIGHMOOR" PEAT PROFILES IN MAINE

THE EFFECT OF SWEET CLOVER AND ALFALFA ROOTS AND TOPS ON THE FUNGOUS FLORA OF THE SOIL

THE GRAVIMETRIC METHOD FOR THE DETERMINATION OF CARBONATES IN SOIL

SOME INFLUENCES OF THE DEVELOPMENT OF HIGHER PLANTS UPON THE MICROÖRGANISMS IN THE SOIL: III. INFLUENCE OF THE STAGE OF PLANT GROWTH UPON SOME ACTIVITIES OF THE ORGANISMS

IS SULFUR A LIMITING FACTOR OF CROP PRODUCTION IN SOME UTAH SOILS

NITRATES IN SOIL AND PLANT AS INDEXES OF THE NITROGEN NEEDS OF A GROWING CROP

THE COMPARATIVE ACID TOLERANCE OF SOME SOUTHERN LEGUMES

A STUDY OF THE NATURE OF THE NITROGENOUS COMPOUNDS IN FUNGOUS TISSUE AND THEIR DECOMPOSITION IN THE SOIL

A. FLOYD HECK

University of Wisconsin[1]

Received for publication August 16, 1928

The decomposition of organic matter in nature, especially the cellulosic portion, is largely accomplished by fungous activities and large amounts of mycelial tissue are formed. The fungous material is relatively high in nitrogen, which is obtained from the substrate, for the most part in the mineral form. As a result, much soluble inorganic nitrogen is changed to an insoluble organic form in the mycelial tissue. The nature of this nitrogen and the factors which govern its return to the inorganic form are made the subject of this study.

OCCURRENCE AND QUANTITY OF SOIL FUNGI

Mold growth in the soil has been observed for a long time. Perhaps Adametz (4) in 1886 was one of the first to isolate molds from the soil. Sixteen years later, in 1902, Oudemaus and Köning (78) isolated and described some 45 species, and thus the first real study of soil fungi began. Following these early workers, the greater part of the investigations with soil fungi has been in relation to systematic studies. Jensen (58), Dale (23, 24), Waksman (109), and Abbott (1, 2, 3) have classified as many as 200 species of fungi living in the soil. Of this large number of species, those of *Penicillium*, *Aspergillus*, *Mucor*, *Trichoderma*, *Cladisporium*, *Fusarium*, and *Rhizopus* are found most often. More recently Gilman and Abbott (41) have compiled a summary of the soil fungi in which is gathered together most of the available material on their classification.

Because of more favorable conditions with respect to oxygen and organic matter, soil fungi are found chiefly in the surface soil. Waksman (108) found few fungi below a depth of 12 to 20 inches, and on the plots at Rothamsted, Brierley (89) reported a rapid diminution in number as the depth increased. On the other hand Rathbun (86) reported fungi to a depth of 44 inches but believed that grubs and worms were in part responsible for this distribution.

[1] From the Departments of Agricultural Bacteriology and Soils. This work was supported in part by a grant from the special research fund of the University of Wisconsin. For the many valuable suggestions and criticisms received in the course of this investigation, the writer wishes to express his sincere gratitude to Dr. E. B. Fred and to Prof. A. R. Whitson, under whose supervision this work was done.

Neither climatic nor soil conditions seem to be deciding factors in the distri bution of fungi, for Pratt (81) isolated many of the lower forms from the desert soils of southern Idaho, and Paine (79) found the same groups to predominate in virgin soils. Geographically, the distribution of soil fungi is almost universal, for Waksman (109) isolated the same species in soils from North America, Europe, and the Hawaiian Islands.

As it is impossible to count the soil fungi *in situ*, their estimation is difficult as well as inaccurate. The dilution method has often been used, but this at best gives only a rough estimate of the number of spores and very little idea as to the actual amount of fungous tissue in the soil. The plate count for soil fungi has been given as ranging from a few thousands to one or two millions per gram of soil, varying with the conditions. Brierley (89) found a sort of seasonal rhythm on the manured plot at Broadbalk, the count being high in March or April and September or October and low in July and December. Waksman (112) showed that the presence of manure or other energy material increased the number of soil fungi. He found a range of 151,000 in a poor forest soil to 525,000 in a garden soil and 750,000 in a meadow soil. Waksman and Starkey (121) have shown that organic matter or energy material added to the soil increases the number of microorganisms. They reported that dextrose increases the number of bacteria, and cellulose the number of fungi in the soil. Rye straw acts very much like cellulose.

Conn (21, 22) and Waksman (112) have shown that fungi exist in the soil in the mycelial form as well as in the spore stage, and McLennan (71) has found that the plate counts are not correlated with the vegetative growth. If the conditions for vegetative growth are good, fewer spores are formed than under more adverse conditions and so the plate count shows a lower number under the better conditions, although in reality a greater quantity of fungous tissue is present. The amount of fungous material present in a soil, therefore, has little relation to the plate count. Brierley (89) estimated that there is about 60 pounds per acre of dry fungous material in the surface soil and that this carries approximately 6 pounds of nitrogen.

ACTIVITIES OF SOIL FUNGI

Relation to energy materials

Fungi are non-chlorophyll bearing plants and must obtain their energy from materials already synthesyzed. This they do by means of enzymes which they secrete. A great many workers (18, 27, 28, 31, 83, 90, 92, 94, 111, 135) have investigated the enzymes of various fungi and have found that practically all of the common organic compounds are hydrolyzed by enzymes secreted by the fungi. The common sugars are readily utilized. It has been shown by several investigators (25, 45, 46, 64, 80, 87, 93) that the pentose sugars and the pentosans in organic materials are used as sources of energy by various groups of the fungi. Cellulose is one of the more resistant substances found

in nature and was originally believed to be hydrolyzed microbiologically only by the bacteria. A number of later workers (46, 48, 50, 69, 75, 91, 103, 118, 120) have shown, however, that cellulose, either in the pure form or in the natural or crude state found in plant residues such as straw, roots, or bagasse, is hydrolyzed by filamentous fungi and used by them as a source of energy. It has also been shown (118) that nitrogen is necessary in this process for the metabolism of the organisms. Lignin is probably one of the most resistant substances found in organic residues. Waksman and Tenney (119) found the lignin unattacked by most soil microorganisms and were able to recover it almost quantitatively at the end of a 35-day incubation period. On the other hand, von Schrenk (94) has shown that even lignin is hydrolyzed and destroyed by the white rot fungus *Polyporus juniperinus*. Other substances such as β-methylglucoside (36) and higher alcohols (76) have been shown to be used as sources of energy by fungi.

Natural organic materials in the form of plant residues probably contain many if not all of these carbonaceous substances which are used as energy for the growth of the fungi. Wheat straw, for example, according to Waksman (113), contains 21.67 per cent pentosans, 34.27 per cent cellulose, and 21.21 per cent lignin. It also contains water-soluble carbonaceous materials, and so furnishes an excellent source of energy for the fungi.

Relation of fungi to nitrogen

Soil microorganisms are concerned with nitrogen in three ways: first, the assimilation of free nitrogen; second, the transformation of organic to inorganic forms; third, the use of combined nitrogen in the production of cellular or mycelial substances.

Fixation of free nitrogen. Among the early workers on the fixation of free nitrogen by soil fungi was Lipman (66) who reported a weak fixing power for species of *Aspergillus* and *Penicillium*. Later workers (17, 42, 63), however, seem to be of the opinion that with the possible exception of *mycorrhiza*, there is no fixation of atmospheric nitrogen by soil fungi. Although Duggar and Davis (37) concurred in the general opinion of the latter workers their work seems to indicate that *Phoma betae* has a weak nitrogen fixing power.

Ammonification. The power of soil fungi to hydrolyze nitrogenous organic materials, especially the proteins and amino compounds found in the organic matter, has been investigated by a number of workers (1, 62, 70, 108, 110, 116) who have shown this property to be very general among the fungi. A great many of the fungi have the power to break down the proteins and to liberate amino acids, or ammonia, or both. Thus the soil fungi play a very important rôle in this stage of the mineralization process.

Use of nitrogen. It is generally conceded by most investigators (9, 12, 19, 60, 82, 118) that nitrogen is not only used by the fungi in their growth but is also a necessary element in the formation of their substance. This nitrogen they may use in the inorganic form as nitrate, nitrite, or ammonia, or in the

various amino forms. Reduction of nitrogen has been shown, but there is very little evidence of denitrification or the loss of combined nitrogen from fungous activities. The use of nitrogen by fungi is always associated with the use of energy material and it has been pointed out (8, 16, 19, 48, 87, 98, 120) that this nitrogen, if not an absolute necessity in the decomposition of the cellulosic energy material, is an aid in this process and hastens the utilization of the cellulose by the fungi. The amount of nitrogen necessary in this decomposition process has been estimated by Waksman and Heukelekian (47, 117, 118) to be 1 part of nitrogen for every 30 to 50 parts of energy material in the form of cellulose used by the fungi. This nitrogen is used in the building of mycelial tissue, and the amount consumed will vary somewhat with the organisms and its environmental conditions.

The result of this use of nitrogen in relation to the utilization of energy material by microorganisms is seen under field conditions in the depression of nitrate nitrogen when the energy material of the soil is increased. A number of workers (5, 68, 74, 85, 95, 97, 99) have reported that when energy material in the form of plant residue, either tops or roots, is incorporated into the soil there is an immediate depression in the nitrate content. At first toxins were thought to be the cause of this depression but later investigations (13, 20, 67, 106) seem to indicate that it is only a disturbance of the energy-nitrogen balance and that the nitrogen is not lost, but is used by the soil microorganisms.

As a brief summary it may be stated that the soil fungi use the carbonaceous materials of the soil as energy for their growth and the soil nitrogen in building their mycelial tissue. If the ratio of energy material to nitrogen is decreased, soluble nitrogen is liberated, but if this ratio is increased, these organisms use up available nitrogen. It is then easy to believe that soil fungi are largely responsible for the rapid depression of mineral nitrogen when the energy supply of the soil is increased.

COMPOSITION OF FUNGOUS MYCELIUM[2]

The composition of fungous tissue has been the subject of investigation for over a century. Perhaps the outstanding single contribution is the monograph by Zellner (136) in which he has compiled much of this material, especially from the earlier works. The greater part of this information, however, is of a general qualitative nature and only a limited amount of quantitative data is given. The reason for this may be seen in the fact that not only the quantitative but even the qualitative composition of fungous tissue depends much upon the environmental conditions of the organism during its period of growth. Zellner has shown that as a group the fungi contain about the

[2] While this article was in press, a paper appeared by R. C. Thomas [Composition of fungus hyphae I. The *Fusaria*. *Amer. Jour. Bot.* 15: 537–547. (1928)] dealing with the composition of the hyphae of species of *Fusaria*. This author thinks that the outer covering of the hyphae is made up of a protein-pectic compound and a cellulose-fatty-acid complex with a basic skeleton of chitin.

same groups of compounds, with the possible exception of cellulose and lignin, as the higher plants. In the nitrogenous group they contain proteins of various kinds, amino acids, amines, basic nitrogenous and purine substances, urea, and lecithin, as well as certain toxic substances of an alkaloid nature. In the carbonaceous group may be mentioned glucose, trehalose, glycogen, pentosans, mycodextran, paraisodextran, inulin, viscosin, chitin, organic acids, fats, higher alcohols, and a cellulosic carbonaceous material of unknown composition.

Nitrogenous portion

Because of variability in culture media, methods of analysis and species employed, the analytical data available for fungous tissue are more or less unsatisfactory from a quantitative standpoint. The carbon content of fungous tissue is rather constant but its content of nitrogen is quite variable. Analyses reported by Sieber (96), Mazé (73), Peterson, Fred, and Schmidt (80), Heukelekian and Waksman (48), and the writer are given in table 1.

These data indicate that the ratio of carbon to nitrogen of the mycelium, although rather stable and usually falling between 7 and 10 to 1, depends somewhat on the carbon-nitrogen ratio of the medium upon which the mycelium is grown and may be greater than 20 to 1 where this ratio in the medium is very high. Waksman and Heukelekian (118) reported 45 per cent of carbon in the dry mycelium and a nitrogen content of from 4 to 8 per cent depending on the nitrogen content of the medium. From the results given, it appears that the nitrogen content of the mycelium decreases with a decrease in the nitrogen supply until it reaches about 2 per cent, after which a further decrease in the supply of nitrogen results in a decreased production of mycelium. The results of other workers are in accord with the data already given.

The nature of the nitrogenous substances in fungous tissue is not definitely known. The following estimates made by Winterstein and Reuter (132) on the tissue of *Boletus edulis* give some idea as to the kinds and amounts of the various substances:

	per cent
Moisture	10
Ether extract	4
(Fat 3.25 per cent, colesterin 0.5 per cent and lecithin)	
Alcohol extract	12
(Trehalose 3 per cent, sugar, lecithin, trimethyl-histidine, adenine, guanine, hypoxanthine, choline, alanine, leucine, purine bodies, bases, etc. 9 per cent)	
Water extract	28
(Glycogen 5 per cent, trehalose, purine bodies, bases, amino acids, ash, etc., 23 per cent)	
Residue	46
(Protein 30 per cent, amorphous carbohydrates [paraisodextran] 10 per cent, and chitin 6 per cent)	

In *Agaricus campestris*, Winterstein and associates (133) estimated that 51.9 per cent of the total nitrogen is protein nitrogen, 7.8 per cent basic nitro-

TABLE 1

Relation of carbon and nitrogen in fungous tissue

INVESTIGATOR	ORGANISM	CULTURE AGE	MEDIA			MYCELIUM		
			Energy material	Nitrogen material	C/N ratio	Carbon	Nitrogen	C/N ratio
		days				*per cent*	*per cent*	
Sieber (1881)	*Penicillium* sp. and *Aspergillus glaucus*	75	Sugar	Gelatin	4.75	45.95	5.32	8.64
	Penicillium sp. and *Aspergillus glaucus*	75	Sugar	NH_4Cl	9.60	46.03	5.34	8.62
Mazé (1902)	*Eurotiopsis gayaui*	5	Sucrose	.....	21.05	51.67	4.48	11.30
	Eurotiopsis gayaui	9	Alcohol	.....	9.01	50.45	5.55	9.10
	Eurotiopsis gayaui	8	Glycerol	.....	19.55	48.89	4.67	10.47
	Eurotiopsis gayaui	8	Lactic acid	.....	20.00	51.51	4.73	10.89
Peterson, Fred and Schmidt (1922)	*Aspergillus niger*	7	Xylose	NH_4NO_3	22.86	44.5	4.5	9.90
	Aspergillus niger	9	Xylose	NH_4NO_3	22.86	46.9	4.6	10.20
	Aspergillus niger	14	Xylose	NH_4NO_3	22.86	46.0	4.2	10.95
	Aspergillus niger	28	Xylose	NH_4NO_3	22.86	45.4	4.2	10.81
	Aspergillus sp.	14	Xylose	NH_4NO_3	22.86	47.3	4.7	10.07
	Penicillium glaucum	9	Xylose	NH_4NO_3	22.86	50.7	5.7	8.90
	Penicillium glaucum	14	Xylose	NH_4NO_3	22.86	46.3	5.0	9.26
	Penicillium glaucum	29	Xylose	NH_4NO_3	22.86	49.7	5.9	8.42
This work (1928)	*Aspergillus oryzae*	14	Sucrose	NH_4NO_3	6.00	40.48	6.42	6.30
	Aspergillus oryzae	14	Sucrose	NH_4NO_3	30.00	41.10	3.83	10.70
	Aspergillus oryzae	14	Sucrose	NH_4NO_3	150.00	39.55	1.89	20.90
						mgm.	*mgm.*	
Heukelekian and Waksman (1925)	*Trichoderma* sp. in solution	17	Cellulose	$(NH_4)_2SO_4$	10.95	54.90	12.3	4.46
	Trichoderma sp. in solution	24	Cellulose	$(NH_4)_2SO_4$	10.95	78.5	18.2	4.31
	Trichoderma sp. in solution	31	Cellulose	$(NH_4)_2SO_4$	10.95	138.2	22.2	6.22
	Trichoderma sp. in solution	38	Cellulose	$(NH_4)_2SO_4$	10.95	138.3	23.7	5.83
	Trichoderma sp. in sand	7	Cellulose	NH_4NO_3	11.40	128.9	18.9	6.80
	Trichoderma sp. in sand	14	Cellulose	NH_4NO_3	11.40	283.3	27.4	10.30

gen, and 40.3 per cent amino acid nitrogen. Supporting these figures Yoshimura and Kania (134) reported 60.26 per cent of the nitrogen in *Cortinellus shiitake* as protein nitrogen, 2.13 per cent as ammonia nitrogen, and 37.61 per cent as non-protein nitrogen.

Besides the usual protein and amino acid compounds, urea has also been reported. Working with the mycelium of *Lycoperdon pyriforme*, Iwanoff (55) found that where the medium on which the organism is grown is high in nitrogenous and low in carbonaceous material, the total nitrogen of the mycelium is high (7.9 per cent) and the urea content may be as high as 4.3 per cent. When the reverse conditions are true the nitrogen content is low (4.3 per cent) and in this case no urea is found in the tissue. With *Lycoperdon molle* he found (51, 52) that the urea content increases very rapidly until just before the ripening stage, when it reaches its maximum, after which there is a sharp decline with usually little or no urea in the mature and ripened tissue. He (56, 57) also showed that *Psalliota campestris* may contain as much as 8.5 per cent of urea and that this organism is able to synthesize urea either from ammonium carbonate or from arginine.

Carbonaceous material

The carbonaceous matter seems to differ somewhat from the carbonaceous substances in ordinary plant materials, in that common sugar, starch, and lignin are not generally reported. Cellulose has been reported but there seems to be some question as to the exact nature of this substance in fungi.

In the tissue of *Boletus edulis*, Winterstein and Reuter (132) have reported trehalose, glycogen, and an amorphous carbohydrate, which they called "paraisodextran." Dox and Neidig (34) treated the mycelium of *Penicillium expansum* with hot water and extracted a polysaccharide which precipitated out as a white powder on cooling. This substances gives d-glucose on hydrolysis and seems to have the same constitution as starch or cellulose but is not hydrolyzed by amylase. They called this substance "mycodextran" and expressed the opinion that it acts as a reserve carbohydrate when the sucrose in the medium is exhausted. Dox (30) showed that with *Aspergillus niger* the content of mycodextran reaches its maximum between 5 and 7 days, or when autolysis begins, at which time it may be as much as 4.5 per cent or more. Dox and Neidig (35) isolated another polysaccharide from the mycelium of *Aspergillus niger* which on hydrolysis gave galactose. This they called "mycogalactan."

The five carbon compounds as a group seem to be found in only very small quantities. Wichers and Tollens (125) studied 10 species of wood-destroying fungi and reported pentosans ranging in amounts from 2.61 to 6.73 per cent by Kröber's method. Ishida and Tollens (49) studied five species and reported pentosans from 2.58 to 5.11 per cent. Dox and Neidig (33) worked with six *Aspergillus* and *Penicillium* species and found that the pentosans in no case exceed 1.17 per cent, which is much lower than for the higher forms

reported by Wichers and Tollens. Schmidt, Peterson, and Fred (93) reported only about 1 per cent of pentosans in molds and found very little variation in the amount whether the molds were grown on xylose or sucrose, whereas Rege (87) found 7.82 per cent in the aerial portion of a *Coprinus* sp. grown on rice straw.

Cellulosic material

The cellulosic portion of fungous mycelium has been studied for more than a century and yet little is definitely known as to its chemical constitution. As early as 1811 Braconnot (15) subjected the mycelium of certain of the edible fungi to lixiviation and to the cellulosic residue he gave the name "fongine." Various attempts were made to identify this material with true cellulose. In 1859 Frémy (40) showed that in many species of fungi the cellulosic material is not soluble in Schweitzer's reagent, which dissolves pure cellulose; he therefore gave to this material the name "metacellulose." As methods were developed for the identification of cellulose it became more and more apparent that this cellulosic material which makes up such a large part of the fungous tissue is not cellulose but a somewhat similar cellulosic complex. After over three-quarters of a century of research along this line it remained for Winterstein (127, 131) to show that this cellulosic complex contains nitrogen. His preparations from fungous tissue of various species, which he carefully freed from nucelin and albumen by treatment with caustic potash and Schulze's reagent, contained from 2.5 to 3.9 per cent nitrogen. On hydrolysis with 3 per cent sulfuric acid he (128) obtained d-glucose, acetic acid, and an undetermined nitrogenous organic substance. Later (129) he determined the combination of nitrogen in this substance by boiling with concentrated hydrochloric acid and obtained a chitosamine hydrochloride, $C_6H_{11}O_5NH_2 \cdot HCl$, which he called a glucosamine. He (130) finally isolated this chitinous material from *Agaricus campestris* and found its nitrogen content to be 6.24 per cent, a value agreeing fairly well with 6.01 per cent given for animal chitin, although in an earlier work (128) he reported nitrogen contents of this material of less than 1 per cent in some cases and with many determinations ranging around 3 per cent. In working with *Boletus edulis*, Reuter (88) found a nitrogenous residue which gave no protein reaction but proved to be a glucosamine or chitinous substance. He also found a carbohydrate like hemicellulose.

Chemists are prone to treat this chitinous material as a definite chemical compound and various formulas have been proposed for it. More recent workers (14, 59) have determined that when a purified product from crab shells is hydrolyzed, it yields nitrogen, acetic acid, and glucose in the ratio of 1:1:1. The latter authors have proposed that it is a polymer of four molecules of mono-acetyl-glucosamine, having a formula of $C_{32}H_{54}N_4O_{21}$, with the amino group joined to the glucose molecule on the one hand and to the acetyl group on the other. Whether this substance is always a definite chemical compound with one amino group to each glucose molecule or whether the nitrogen may

be more or less variable, being replaced in many cases by the -OH group of the glucose as it is in cellulose, is not definitely known. It would seem from the cellulosic nature of this substance that it may vary from the formula given above for chitin, which has a glucose-nitrogen ratio of 1:1, to that of cellulose which contains no nitrogen. The variable results of early workers and the variable nitrogen figures given by Winterstein point very strongly to the latter view.

Little is known regarding the amount of this cellulosic material. Unpublished work done at the Forest Products Laboratories, Madison, Wisconsin, indicates that about half of the dry weight of the mycelium is left after the process of chlorination, but nothing definite is known as to the constitution of this material. Tanret (102) reported 15 per cent of chitin in the mycelium of *Aspergillus niger;* Proskuriakow (84), 5.5 per cent in the fruiting bodies of *Agaricus campestris;* and Winterstein and Reuter (132) found 6 per cent of this material in the tissue of *Boletus edulis.* From the fruiting bodies of *Lycoperdon pyriforme,* Iwanoff (54) isolated an alcohol-insoluble material which yields glucosamine on hydrolysis. He (53) also reported viscosin from the same organism in amounts varying from 11 to 26 per cent. This material contains from 6.1 to 6.5 per cent of nitrogen and gives glucosamines on hydrolysis. He considered this material along with the chitosans as an intermediate product in the synthesis of chitin from glucose.

DECOMPOSITION AND NITRIFICATION OF FUNGOUS TISSUE

Since the mycelial tissue of soil fungi probably makes up a large part of the microbial materials of the soil, the decomposition of this material and especially the change of its nitrogen to the inorganic form is a very important process in relation to soil fertility. Since the saprophytic soil fungi are unable to fix free nitrogen they must obtain this element from the combined nitrogen of the soil, either from solution or by the hydrolysis of organic nitrogenous compounds. In the use of soil nitrogen the fungi have a distinct advantage as compared to the higher plants in that the fungi are able to assimilate their nitrogen before it reaches the nitrate stage, which most green plants are unable to do. The fungi absorb the mineral soil nitrogen, quickly converting it into organic form, and thus it is removed from the sphere of availability for higher plants.

Whether this nitrogen which has been stored up in the tissues of these organisms is again readily available for higher plants or whether it is more or less resistant and unavailable has been given little attention by research workers and very little is reported on the decomposition of fungous material. Starkey (100) used the evolution of carbon dioxide as an index of the decomposition of organic matter in the soil and found that on decomposition a fungous material containing 3.84 per cent of nitrogen and 44.3 per cent of carbon evolves carbon dioxide to a greater extent than alfalfa meal and almost equal to dried blood. The alfalfa meal in this case contained 2.45 per cent of nitrogen and 40.62 per cent of carbon, and the dried blood 9.61 per cent of nitrogen and

37.52 per cent of carbon. He reported 67 per cent of the carbon of the fungous material lost in 10 days. When nitrate nitrogen is added the decomposition is accelerated. Rege (87) found that when the mycelial tissue of a *Coprinus* sp., which contained 3.5 per cent of nitrogen, was placed in the soil at the rate of 55 p.p.m. of nitrogen, the yields of mustard were inferior to those where dried blood and ammonium sulfate containing equal quantities of nitrogen were used, and very little greater than the control. His figures are as follows:

Control	3.85 gm. mustard (dry)
Ammonium sulfate	7.40 gm. mustard (dry)
Dried blood	7.10 gm. mustard (dry)
Coprinus sp.	4.70 gm. mustard (dry)

This work indicates that the nitrogen in this fungous tissue does not readily respond to the process of nitrification and is therefore not easily made available for plant use, although this material contained 3.5 per cent of nitrogen, a figure rather high for a green manure. Falck (38) showed that the nitrogen in fungous tissue is not available for the use of flax, oats, or peas but that young pines are able to use this nitrogen, perhaps because of their association with mycorrhiza fungi. In his work, manure containing much energy material was present in the sand cultures, which may help to explain his results.

In contrast to this work, Barthel and Bengtsson (10) very recently report that the mycelium of *Aspergillus niger* is readily decomposed and nitrified in the soil. Bierema (12) reported that from 0 to 40 per cent of the nitrogen in the tissue of *Penicillium glaucum* and *Mucor racemosus* is changed to nitrate in two months. He thinks that the older the culture and the more spores present, the slower the nitrification. The process of sterilization seems to decrease the rate of nitrate accumulation.

No attempt is made to review all the literature dealing with fungi, but only that which applies particularly to the subject at hand. Lafar (65), Russell (89), Waksman (115), and Zellner (136) have each given good reviews of various phases of the literature on fungi.

EXPERIMENTAL WORK

SOURCES OF FUNGOUS MATERIAL

Collection of fungous tissue from natural habitats

Large quantities of fungous tissue were necessary for the decomposition studies and also for chemical analyses. Many species were used in order that conclusions might not be drawn from insufficient data.

Attention was first directed to the many forms of the higher fungi found growing in nature and commonly known as mushrooms. Collections were made during September, October, and November. These materials were all of the higher fungi, and the ones collected were mainly the gill forms. The tissue obtained was only the aerial portion, consisting of the stipe and the pileus

of the fruiting body. It was impossible to obtain the mycelial tissue or hyphae from the substrate in quantities sufficient for either chemical or nitrification studies.

The fruiting or aerial portions were gathered, and while still fresh were separated from all foreign matter and washed with distilled water. They were then divided into small parts, placed on trays with screen bottoms, and the temperature quickly raised to 60°C. for a short time to stop the action of enzymes. The temperature was then lowered to about 40°C. and the material was quickly dried by means of an air current. It was later dried at 65°C. for 48 hours and ground to pass a 40-mesh sieve. In two cases the stipes were separated from the pilei.

The following species were collected from their natural habitats and the figures give the approximate amounts of dry material obtained:

	gm.
1. *Secotium acuminatum* Mont.	500
2. *Marasmius oreades* (stipes)	33
3. *Marasmius oreades* (pilei)	75
4. *Lycoperdon pyriforme*	240
5. *Coprinus* sp. (stipes)	140
6. *Coprinus* sp. (pilei)	60
7. *Pholiota adiposa*	100
8. *Clitocybe multiceps*	310
9. *Fomes igniarius*	200
10. *Polyporus graveolens**	80

* Species is questionable.

These species are all members of the higher group of fungi and were collected during the rainy fall months. They were all found growing where there was a more or less abundant supply of cellulosic energy material in the substrate. The *Pholiota*, *Fomes*, and *Polyporus* species were collected from the dead stumps of trees. The other species were found growing either on the ground or on manure. The *Coprinus* sp. is one of the inky cap mushrooms and was collected from a manure heap. The *Marasmius oreades* is the fairy ring fungus which grows in more or less complete rings on the lawn. The rings range from 1 or 2 to 10 or 12 feet in diameter. The *Secotium acuminatum* was found growing on an earth-covered compost heap. The fruiting bodies were numerous and as large as three inches in diameter. The *Clitocybe multiceps* and *Lycoperdon pyriforme* were found growing on pieces of decaying wood in the surface soil. All of these species were produced on a substrate low in available nitrogen and high in cellulosic energy material.

Attempts were made to produce the higher forms on large artificial manure cultures. Large outdoor bins were packed with 1000 pounds of straw and treated with lime; lime and ammonium sulfate; lime, ammonium sulfate, and acid phosphate; and lime and legume hay. Large steel tanks were set up indoors and 100-pound quantities of chopped straw were treated in the same way. These artificial manure cultures produced a number of *Coprinus* sp. but not in sufficient quantities to be of any value.

Production of fungous tissue on synthetic liquid media

Production in 1926. Pure cultures of *Aspergillus oryzae* were first grown on a modification of Pfeffer's solution as described by Steinberg (101). This medium was further modified to give three culture solutions of widely different carbon-nitrogen ratios. The culture solutions were made up by adding water and varying amounts of ammonium nitrate to a stock solution.

Stock solution

Sugar (sucrose)	100 gm.
Mono-potassium phosphate	10 gm.
Magnesium sulfate	5 gm.
Ferrous sulfate	Trace
Zinc sulfate	Trace
Water	1000 cc.

Culture solutions

1. High nitrogen solution (Carbon-nitrogen ratio = 6:1)	
Stock solution	1000 cc.
Ammonium nitrate	20 gm.
Water	1000 cc.
2. Medium nitrogen solution (Carbon-nitrogen ratio = 30:1)	
Stock solution	1000 cc.
Ammonium nitrate	4 gm.
Water	1000 cc.
3. Low nitrogen solution (Carbon-nitrogen ratio = 150:1)	
Stock solution	1000 cc.
Ammonium nitrate	0.8 gm.
Water	1000 cc.

Fifty-cubic centimeter portions of these solutions were placed in 12-ounce prescription bottles and sterilized at 15 pounds pressure for 30 minutes. They were then seeded with spores of *Aspergillus oryzae* and incubated in a horizontal position for 14 days at which time the pads were harvested. After being drawn from the bottles, the pads were placed in cold water and washed for an hour in several changes of tap water and finally in two or three changes of distilled water to free them from as much water-soluble material as possible. No doubt there was some loss of organic material from the broken hyphae. The pads were then dried at room temperature for 12 hours and finally in an oven at 37°C. This drying did not remove all of the water, for from 3.34 to 6.25 per cent remained. The material was then ground to pass a 60-mesh sieve.

The differences in the character of growth on the three solutions were very marked. The high nitrogen pads produced practically no spores, the medium nitrogen cultures fruited rather well, whereas those growing on the low nitrogen medium fruited profusely and rather early. In each case at the end of 14 days all of the sugar in the culture solution was consumed.

Two species of the higher fungi, *Coprinus radians* and *Psilocybe atamatoides*,

were grown on a 6 per cent (by weight) malt extract medium made up from a malt extract having the following composition:

	per cent
Maltose	51.02
Dextrose	10.94
Albuminate	3.11
Nitrogen (Kjeldahl)	0.95
Glycerine	9.27
Free acid	0.43
Inorganic substance	1.16
Water	22.47

The mycelium was grown, washed, dried, and handled in much the same way as was that of the *Aspergillus oryzae*. This particular *Coprinus radians* will be designated as *Coprinus radians* (*1926*) to distinguish it from that later grown on a malt-sugar solution.

Production in 1927.—In later attempts to produce pure cultures of fungi on liquid media so that the entire mycelial structure could be obtained, it was found that the higher forms grew very poorly on any synthetic medium tried, and that best growth was secured with a malt solution.

The lower forms, however, grew well on synthetic media, but each species of organism has its own particular requirements in the solution medium. After many trials a modification of the peptone nutrient medium of Klotz (61) was used for the production of *Trichoderma lignorum* and *Aspergillus oryzae*.

Modified peptone nutrient medium

Sucrose	30 gm.
Mono-potassium phosphate	5 gm.
Ammonium nitrate	2 gm.
Peptone	5 gm.
Magnesium sulfate	0.2 gm.
Calcium sulfate	0.2 gm.
Ferrous sulfate	Trace
Sodium chloride	Trace
Phosphoric acid	to pH 5
Water	1000 cc.

The *Trichoderma lignorum* grown on this medium produced an average dry pad of about 590 mgm. per 50 cc. of solution. On this medium, growth was normal and very good, giving a thick pad with only light sporulation. On the contrary Pfeffer's solution used in earlier work produced a very light pad and sporulated profusely. The *Aspergillus oryzae* did not grow so well on this solution as on Pfeffer's. The average weight of dry pad was only about 400 mgm. per 50 cc. of solution. This particular tissue of *Aspergillus oryzae* is designated as *Aspergillus oryzae* (*1927*).

For the growth of the higher forms a malt-sugar solution was used. The

analysis of this malt extract has been given and the composition of the solution was as follows:

Malt extract	30 gm.
Sucrose	10 gm.
Water	1000 cc.

On this solution were grown *Coprinus radians* and a *Coprinus* sp. (isolated from manure). The former species produced nearly a normal growth with some normal fruiting bodies and gave a dry pad weight of about 350 mgm., whereas the latter grew very poorly and gave a dry pad weight of between 180 and 200 mgm. per 50 cc. of solution.

This study seemed to indicate that if the fungi were grown on liquid media, a special solution would have to be found for each organism, as no two seem to give good growth with the same medium. Each solution with each organism produces its own particular pH reaction.

After removal from the medium the mycelial tissue was first washed and then dried, as previously explained, and ground to pass a 40-mesh sieve. Dry tissue of the four organisms grown in pure culture was obtained in the following approximate amounts:

	gm.
Trichoderma lignorum	100
Aspergillus oryzae (1927)	75
Coprinus radians (1927)	35
Coprinus sp.	20

CHEMICAL METHODS—SEPARATIONS

Water-soluble and -insoluble fractions

To secure the water-soluble and water-insoluble fractions of the fungous tissue and other organic matter, they were shaken with 60 cc. of water per gram of material and allowed to stand at room temperature with frequent shakings for 24 hours. They were then decanted through a C. S. & S. 589 white ribbon filter and washed with 40 cc. of water per gram used. Since the amount of filterable material depends much upon the grade of filter and method of filtering, this procedure was used in all separations for culture work and for the analysis of the culture material.

Dialization

The dializable portion was obtained by placing 3 gm. of the dry fungous tissue in a collodion sack with 25 cc. of water. The sack was then closed and suspended in a cylinder containing 150 cc. of water, and gently rotated. The water was changed four times during a 24-hour period, and the total solution taken off contained the dializable portion.

The contents of the sack were then placed in a beaker and diluted to 150 cc. with water, and after standing at room temperature a few hours were filtered

by suction through filter pulp on a Büchner funnel. The filter pulp and residue were returned to the beaker and this process was repeated twice. The filtrate contained the non-dializable portion of the water-soluble material.

Alcohol- and alkali-soluble and -insoluble fractions

It was found impracticable to use aqueous solutions, especially of sodium hydroxide, where both the filtrate and the residue were to be saved. To obviate this difficulty a 60 per cent alcoholic solution was used, and this was found to filter rather easily through hardened filter paper (C. S. & S.), with suction, both with the alcohol alone and with 0.05 *N* sodium hydroxide. Three grams of the material was digested at 50°C. in 100 cc. of the solvent for about 6 to 12 hours, filtered, and washed once or twice. It was then returned to the beaker and again digested in this way twice more, the whole process covering a 24-hour period. The filtrates were placed together and concentrated to volume.

The residue from the 60 per cent alcohol was then treated in the same way with 0.05 *N* sodium hydroxide in 60 per cent alcohol, giving in all three fractions, the alcohol-soluble, the alkali-soluble, and the residue, which were used for analysis.

ANALYTICAL METHODS

Total nitrogen. A modification of the Kjeldahl-Gunning method for total nitrogen was used in which mercuric oxide was the catalyst and sodium thiosulfate introduced before distillation for the precipitation of the mercury.

Ammonia nitrogen. Determinations were made by Harper's (43) method of extracting the wet soil with 10 per cent potassium chloride solution and subsequent distillation with heavy magnesium oxide. The ammonia was absorbed in an excess of standard acid and after the distillate had been boiled and cooled the excess acid was titrated to neutrality with standard alkali, using methyl red as the indicator.

Nitrate nitrogen. Harper's (44) modified phenol-di-sulfonic acid method was used with slight changes. The soils were dried at 65°C. for 24 hours to eliminate the water factor; alum and a saturated solution of calcium hydroxide were used to secure flocculation.

Amino nitrogen. Van Slyke's (104, 105) method was used as outlined by Hawk. The apparatus used was equipped with a micro-pipette.

Urea. A modification of Marshall's (72) clinical method for urea was used. Methyl red was substituted for methyl orange, the solution made slightly acid with standard acid and after boiling, the excess was titrated with standard sodium hydroxide.

Total carbon. Carbon was determined by the wet combustion method as given by the Association of Official Agricultural Chemists (7). The apparatus used was a very simple modification of that used by White and Holben (122) in their perfected method. This apparatus will be described in another paper.

The carbon dioxide was absorbed in 0.5 *N* sodium hydroxide, precipitated as the carbonate with neutral 2 *N* barium chloride and the excess sodium hydroxide titrated with 0.5 *N* hydrochloric acid, using phenolphthalein as the indicator.

Carbon dioxide carbon. The carbon dioxide was absorbed and estimated as outlined for total carbon. Continuous aspiration over the surface of the soil was employed.

ANALYSIS OF FUNGOUS TISSUE AND OTHER MATERIALS

Materials grown in 1926

The carbon and nitrogen contents of the fungous mycelia produced in 1926 compare favorably with those given in table 1. The analyses of mycelium of

TABLE 2

Composition of pure cultures of Aspergillus oryzae (1926), Psilocybe atamatoides and Coprinus radians (1926)

Air dry material

	ASPERGILLUS ORYZAE (1926)			PSILOCYBE ATAMATOIDES	COPRINUS RADIANS (1926)
	High*	Medium*	Low*		
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Moisture	6.25	4.25	3.34	0.71	1.11
Ash	7.05	7.19	9.25	3.39	4.97
Total carbon	40.48	41.10	39.55	....	....
Total nitrogen	6.42	3.83	1.89	2.26	3.21
Water-soluble nitrogen	3.46	1.70	0.89	1.07	1.65
Average weight of pad, mgm.	672	653	290	....	....
Carbon-nitrogen ratio of the tissue	6.3	10.7	20.9	....	....

* Refers to the nitrogen content of the culture medium. The ratio of the carbon to nitrogen in the high nitrogen medium was 6 to 1, in the medium nitrogen medium, 30 to 1, and in the low nitrogen medium 150 to 1.

Aspergillus oryzae grown on Pfeffer's solution and of *Coprinus radians* and *Psilocybe atamatoides* grown on a 6 per cent malt extract solution are given in table 2.

These data indicate three points that are borne out in subsequent work; (*a*) That the carbon content of the tissue is approximately constant, (*b*) that the nitrogen content varies inversely, and the ratio of carbon to nitrogen in the tissue directly, with the carbon-nitrogen ratio of the medium, and (*c*) that roughly half of the nitrogen in the dry fungous tissue is soluble in water.

The relation of the nitrogen in the substrate to that in the fungous tissue is significant. Vorbrodt (107) found that on a medium containing 1 per cent of ammonium nitrate, mycelium of *Aspergillus niger* containing 7 per cent of nitrogen is produced. But when the ammonium nitrate in the medium is

reduced to 0.05 per cent the mycelium contains only 2 per cent of nitrogen. In table 2 the high nitrogen medium contained 1 per cent and the low nitrogen medium 0.04 per cent of ammonium nitrate. These have produced mycelial tissue of *Aspergillus oryzae* with approximately the same nitrogen contents as the tissues of *Aspergillus niger* reported by Vorbrodt. These data show that where the ratio of carbon to nitrogen is between 6 and 30 to 1, the weight of the tissue produced on the same amount of medium is approximately the same but more nitrogen is assimilated by the organisms from the high nitrogen medium. As the carbon-nitrogen ratio of the medium is raised above 30 to 1 the weight of the mycelium decreases much faster than the nitrogen in the tissue, and a minimum nitrogen content of about 2 per cent is maintained. There is little drop in the weight of the mycelial tissue until its ratio of carbon to nitrogen reaches approximately 10 or 12 to 1. In this respect the fungi are much like the higher plants, as they are able to adjust their growth and nitrogen content to the supply of available nitrogen.

The nitrogen of the dry mycelial tissue is approximately 50 per cent soluble in water. With the *Aspergillus oryzae* there is a tendency for the tissue of highest nitrogen content to have the greatest solubility. Vorbrodt found the same to be true of *Aspergillus niger*.

Materials for carbon evolution and plant culture studies

Tables 3, 4, and 5 give the percentages of carbon and nitrogen in all of the materials used in the experimental work on the evolution of carbon dioxide and nitrate accumulation in soils and also in the plant culture work. Here again it will be noted that the carbon content of the materials is rather constant, with few exceptions falling between 41 and 44 per cent. As with the *Aspergillus oryzae* in table 2, the nitrogen content and the ratio of carbon to nitrogen vary greatly, depending on two factors, the carbon-nitrogen or rather the energy-nitrogen ratio of the substrate and the species of the organism. This variation of nitrogen content is not only true in the forms grown on synthetic media but is also true of the forms collected from their natural habitats. It is remarkable that the puffball, *Lycoperdon pyriforme*, growing on a piece of rotting wood in a very poor soil, should have a nitrogen content of over 5 per cent. The same is true of *Marasmius oreades*, which, growing in competition with lawn grass, has a nitrogen content of about 7 per cent.

As compared with plant material, most of the fungous tissue is high in nitrogen even though the tissue is produced on substrate of low nitrogen content. Although the pilei of the higher fungi contain more nitrogen than the stipes, the latter are higher in that element than ordinary plant tissue. The high nitrogen content of the mycelial tissue of the fungi indicates a rapid assimilation of nitrogen and this together with the fact that the fungi are able to use their nitrogen in the ammonia form makes it practically impossible for green plants to assimilate soil nitrogen when energy material and fungi are present in the soil.

The water solubility of the fungous nitrogen varies greatly with the species and with the substrate upon which the tissue is produced. The solubility of the nitrogen in water varies from a little less than half, in the *Aspergillus*

TABLE 3

Carbon and nitrogen content of organic materials and fungous tissues

Air-dry material

MATERIAL	CARBON	NITROGEN
	per cent	*per cent*
Ammonium sulfate	....	21.05
Blood meal	46.8	14.74
Cottonseed meal	44.6	6.64
Alfalfa hay	43.7	2.58
Timothy hay	44.5	1.19
Straw (*mixed grain*)	44.4	1.04
Saccharomyces cerevisiae	43.8	9.53
Trichoderma lignorum	44.4	5.13
Aspergillus oryzae (1927)	41.9	4.58
Aspergillus oryzae (1926 Med. N)	41.1	3.83
Coprinus radians (1927)	41.2	2.27

TABLE 4

Carbon and nitrogen content of higher fungi collected from their natural habitats

Air-dry material

MATERIAL	CARBON	NITROGEN
	per cent	*per cent*
Secotium acuminatum	41.2	7.71
Marasmius oreades (pilei)	42.4	7.10
Marasmius oreades (stipes)	41.8	6.37
Coprinus sp. (pilei)	42.2	6.42
Lycoperdon pyriforme	42.5	5.04
Coprinus sp. (stipes)	38.8	4.00
Pholiota adiposa	41.7	3.84
Clitocybe multiceps	40.6	3.74
Fomes igniarius	44.3	1.71
Polyporus graveolens	41.7	1.56

oryzae (1926 medium nitrogen), to about five-sixths, in the *Clitocybe multiceps*. With cottonseed meal only a small part is water-soluble and with alfalfa hay approximately two-fifths is dissolved in water.

Distribution of carbon and nitrogen in fungous tissue

The tissues of *Lycoperdon pyriforme* and *Trichoderma lignorum*, which represent different forms produced under widely different conditions, were used throughout this work. The former is one of the higher forms found growing in nature on rotting wood and the latter is one of the molds or lower forms produced on a synthetic liquid culture medium.

These tissues were separated into fractions by two methods; first, by water solubility, in which the dializable, the water-soluble non-dializable, and the water-insoluble fractions were obtained, and second, by alcohol and alkali solubility, in which the alcohol- and alkali-soluble and the alkali-insoluble fractions were obtained. Tables 6 and 7 give a summary of this work.

TABLE 5

Carbon and nitrogen content of the water-soluble and water-insoluble fractions of various materials

Air-dry material

MATERIAL	CARBON	NITROGEN
	per cent	*per cent*
Cottonseed meal, water-soluble	7.1	0.74
Cottonseed meal, water-insoluble	37.6	5.90
Alfalfa hay, water-soluble	12.9	1.05
Alfalfa, hay, water-insoluble	30.8	1.53
Lycoperdon pyriforme, water-soluble	17.2	2.83
Lycoperdon pyriforme, water-insoluble	25.3	2.21
Lycoperdon pyriforme, 0.3 per cent NaOH insoluble	18.1	1.72
Trichoderma lignorum, water-soluble	19.7	3.48
Trichoderma lignorum, water-insoluble	24.7	1.65
Clitocybe multiceps, water-soluble	21.4	3.15
Clitocybe multiceps, water-insoluble	19.2	0.59
Clitocybe multiceps, 0.3 per cent NaOH insoluble	15.1	0.60
Aspergillus oryzae (1926 Med. N), water-soluble	5.7	1.73
Aspergillus oryzae (1926 Med. N), water-insoluble	35.4	2.10

In both of the species reported, over half of the weight of the material and from 50 to 70 per cent of the nitrogen is soluble in water, which is in accord with the work of Vorbrodt (107) with *Aspergillus niger*. Of this water-soluble nitrogen less than 10 per cent is non-dializable and approximately half is in the free amino form. These facts indicate that the nitrogen of the water-soluble fraction is made up of very simple proteins and amino acids. The higher water solubility is in the less mature tissue of *Trichoderma* grown on liquid culture medium, and indicates that age and amount of available nitrogen affect the solubility of the nitrogen in the tissue.

The alcohol-soluble portion is not so large as the water-soluble fraction but has about the same proportion of amino nitrogen. The alkali-soluble fraction makes up from 45 to 60 per cent of the total nitrogen and is less simple than the water-soluble fraction, being only 10 to 15 per cent amino nitrogen. This

fraction shows more complex proteinaceous materials which are not water-soluble. From 16 to 18 per cent of the nitrogen in the tissue is insoluble in

TABLE 6

Distribution of carbon and nitrogen in the tissue of Lycoperdon pyriforme

Calculated in 1 gm. of air-dry material

FRACTION	WEIGHT OF MATERIAL	CARBON	TOTAL NITROGEN		AMINO-NITROGEN	
	mgm.	*mgm.*	*mgm.*	*per cent*	*mgm.*	*per cent*
Total (original sample)†	1,000	425	50.4			
Water-soluble fraction						
Dializable	430	162	24.3	48.2	10.25	42.2
Non-dializable	93.3	31.5	2.62	5.2	1.69	64.5
Water-insoluble fraction*	476.7	231.5	23.5	46.6		
Alcohol-soluble fraction‡	375	140.6	18.0	35.7	9.14	50.8
Alkali-soluble fraction§	148*	92.6	23.12	45.9	3.28	14.2
Alcohol- and alkali-insoluble fraction	451	175.5	9.25	18.3		
Loss		16.3	0.03	0.1		

* By difference.

† Moisture 2.61 per cent.

‡ 60 per cent alcohol.

§ 0.05*N* sodium hydroxide in 60 per cent alcohol after the alcohol-soluble fraction was removed.

TABLE 7

Distribution of carbon and nitrogen in the tissue of Trichoderma lignorum

Calculated in 1 gm. of air-dry material

FRACTION	WEIGHT OF MATERIAL	CARBON	TOTAL NITROGEN		AMINO-NITROGEN	
	mgm.	*mgm.*	*mgm.*	*per cent*	*mgm.*	*per cent*
Total (original sample)	1,000	444	51.3			
Water-soluble fraction:						
Dializable	416.4	177.6	33.17	64.6	15.65	47.1
Non-dializable	97.1	38.0	2.59	5.05	1.31	57.5
Water-insoluble fraction*	486.5	228.4	15.54	30.35		
Alcohol-soluble fraction†	237.6	105	9.2	18.0	5.68	61.7
Alkali-soluble fraction‡		124.2	32.2	62.8	2.96	9.2
Alcohol- and alkali-insoluble fraction	344.8	137.9	8.36	16.3		
Loss		76.9	1.54	2.9		

* By difference.

† 60 per cent alcohol.

‡ 0.05*N* sodium hydroxide in 60 per cent alcohol after the alcohol-soluble fraction was removed.

0.05 *N* sodium hydroxide. This may be reduced somewhat by using stronger alkali, but usually from 12 to 15 per cent of the fungous nitrogen remains in

the alkali-insoluble residue. The form of this nitrogen is not known, but it must be of a more or less complex protein nature with perhaps some chitinous forms. This residual material is no doubt one of the contributing factors mentioned by Waksman (114) in the composition of soil humus.

Although Iwanoff (51, 52, 55, 56, 57) has reported as much as 8.5 per cent of urea in *Psalliota campestris*, and 4.3 per cent in *Lycoperdon pyriforme*, none could be found in either the *Lycoperdon pyriforme* or the *Trichoderma lignorum* used in this work.

On the whole it appears from the data given and from the work of other investigators, that much of the nitrogen in fungous tissue is readily soluble in water and of a simple nature. Although the insoluble residue may contain some chitinous nitrogen, the amount is small and cannot possibly be of any considerable importance in the mineralization of fungous nitrogen.

LABORATORY AND GREENHOUSE WORK ON THE AVAILABILITY OF FUNGOUS NITROGEN

Nitrification studies

It has already been shown that the fungi and particularly those forms living in the soil use much inorganic nitrogen and convert it into organic forms in their tissue. The amount of nitrogen in fungous material is variable and relatively high. It is approximately half soluble in water and largely of simple structure. In order to study the return of this nitrogen to the inorganic form and the factors which govern this mineralization process, three sets of experiments were carried out: first, nitrification studies on fungous tissue of the same species with varying nitrogen contents; second, nitrification studies of various species with a study of the carbon relations; and third, a study of the assimilation of the nitrogen from fungous tissue by higher plants. In the first part, the tissues of *Aspergillus oryzae* (1926), *Coprinus radians* (1926), and *Psilocybe atamatoides* were used.

The air-dried material as well as the living pads as they were removed from the wash water, were placed in 1200 gm. portions of sifted and well-mixed Miami silt loam at the rate of 2 tons (2.4 gm. per pot) of dry material per acre. As this soil was acid (pH 5.8), precipitated calcium carbonate was added at the same rate. The moisture was made up to 20 per cent and held constant. The work was carried out in half-gallon earthenware pots with incubation at 23°C.

The tissue of *Aspergillus oryzae* used in this work was added to the soil in six different forms as follows:

1. Living mycelium; the living tissue on being removed from the wash water was divided into small pieces and placed directly into the moist soil.
2. Living mycelium dried after 20 days; same as no. 1 but after being in the soil for 20 days the entire soil mass was spread out and air-dried for 48 hours. It was then returned to the pot and made up to its original moisture content.
3. Sterilized mycelium; the dry tissue was sterilized at 15 pounds steam pressure for 45 minutes before being placed in the soil.

4. Dry mycelium; the tissue was dried at 65°C. for 48 hours before being placed in the soil.
5. Water-soluble fraction; the water-soluble fraction of no. 4.
6. Water-insoluble fraction; the water-insoluble fraction of no. 4.

In the Aspergillus series there were some variations in the amounts of nitrogen and dry matter where the living materials were added. Whole pads were used

TABLE 8

Number of bacteria per gram of soil according to plate count

TREATMENT PER POT	AFTER 30 DAYS	AFTER 40 DAYS
	millions	*millions*
Controls	16.3	14.4
Controls, dried after 20 days*	8.0	18.8
Aspergillus oryzae, 6.42 per cent N		
Living mycelium, 4 pads	61.6	92.6
Dried after 20 days	.....	92.6
Sterilized mycelium, 2.4 gm	126.0	265.0
Dry mycelium, 2.4 gm	106.0	184.3
Water-soluble fraction	49.0	34.3
Water-insoluble fraction	79.0	105.3
Aspergillus oryzae, 3.38 per cent N		
Living mycelium, 4 pads	59.0	100.6
Dried after 20 days	.....	157.6
Sterilized mycelium, 2.4 gm	132.0	271.0
Dry mycelium, 2.4 gm	101.0	240.3
Water-soluble fraction	27.0	42.3
Water-insoluble fraction	86.3	202.6
Aspergillus oryzae, 1.89 per cent N		
Living mycelium, 8 pads	67.0	109.6
Dried after 20 days	.....	110.6
Sterilized mycelium, 2.4 gm	117.0	176.0
Dry mycelium, 2.4 gm	106.0	245.6
Water-soluble fraction	23.0	41.0
Water-insoluble fraction	92.0	95.0
Wheat Straw		
Finely ground, 2.4 gm	31.0	21.7

* After 20 days, the contents of the pots were removed, air dried for 48 hours, and returned.

and their dry weights were not exactly the same as those of the dry materials. The amounts of nitrogen added are given in table 9.

McLennan (71) has very recently shown that the air drying of fungous tissue kills the vegetative hyphae but that this treatment has no effect upon the spores. When a soil containing living fungous hyphae is air dried, as in the case of no. 2 above, it is reasonable to expect that this tissue will be killed.

TABLE 9

The accumulation of nitrate nitrogen from decomposing fungous tissue in soil

Calculated in 100 gm. of soil

TREATMENT PER POT	NITROGEN ADDED	NITRATE NITROGEN RECOVERED			PERCENTAGE OF NITROGEN RECOVERED		
		20 days	40 days	80 days	20 days	40 days	80 days
	mgm.	*mgm.*	*mgm.*	*mgm.*	*per cent*	*per cent*	*per cent*
Aspergillus oryzae, 6.42 per cent N							
Living mycelium, 4 pads	14.83	8.4	9.5	11.2	56.5	63.8	75.3
Dried after 20 days	14.83	8.5	9.6	10.9	57.3	64.7	73.4
Sterilized mycelium, 2.4 gm.	12.84	4.54	5.3	6.0	35.4	41.2	46.7
Dry mycelium, 2.4 gm.	12.84	6.2	6.8	7.6	48.3	52.9	59.4
Water-soluble fraction	6.92	4.96	5.0	4.93	71.6	72.2	71.3
Water-insoluble fraction	5.92	0.96	1.44	1.8	16.2	24.3	30.2
Aspergillus oryzae, 3.38 per cent N							
Living mycelium, 4 pads	9.14	2.31	3.62	4.17	25.2	39.6	45.8
Dried after 20 days	9.14	2.16	3.00	3.46	23.6	32.8	37.8
Sterilized mycelium, 2.4 gm.	7.66	0.61	1.12	1.50	6.9	14.7	19.4
Dry mycelium, 2.4 gm.	7.66	1.01	1.50	2.50	13.2	19.6	32.7
Water-soluble fraction	3.40	1.96	2.00	2.15	57.7	58.9	63.3
Water-insoluble fraction	4.26	−0.34	0.37	0.66	....	8.8	15.5
Aspergillus oryzae, 1.89 per cent N							
Living mycelium, 8 pads	3.73	−0.62	0.67	1.00	....	17.9	26.7
Dried after 20 days	3.73	−0.67	0.37	0.60	....	9.8	16.1
Sterilized mycelium, 2.4 gm.	3.78	−1.84	−0.77	0.15	....	....	4.0
Dry mycelium, 2.4 gm.	3.78	−1.86	−0.97	−0.25	....	....	....
Water-soluble fraction	1.78	0.81	0.87	1.00	45.4	49.2	56.3
Water-insoluble fraction	2.00	−2.02	−1.43	−0.67	....	....	....
Wheat straw							
Finely ground, 2.4 gm.	0.76	−2.43	−1.92	−1.35	....	....	....
Psilocybe atamatoides							
Dry mycelium, 2.4 gm.	4.52	−1.03	.....	.....	....	....	....
Water-soluble fraction	2.14	0.50	.....	.....	23.3	....	....
Water-insoluble fraction	2.37	−1.22	.....	.....	....	....	....
Coprinus radians (1926)							
Dry mycelium, 2.4 gm.	6.42	1.09	.....	.....	17.0	....	....
Water-soluble fraction	3.30	1.79	.....	.....	54.2	....	....
Water-insoluble fraction	3.12	0.11	.....	.....	3.85	....	....

− means below the control.

Its decomposition will then be the same as that for dead tissue. The data given in tables 8 and 9 and also shown graphically in figures 1 and 2 show the effects of these various treatments upon the nitrification of fungous tissue.

Number of bacteria and amount of fungous growth

After 30 and 40 days, bacterial counts of these soils were made on 0.1 per cent nutrose (sodium caseinate) agar. Table 8 shows the number of bacteria per gram of soil for the various treatments including wheat straw at the rate of 2 tons per acre.

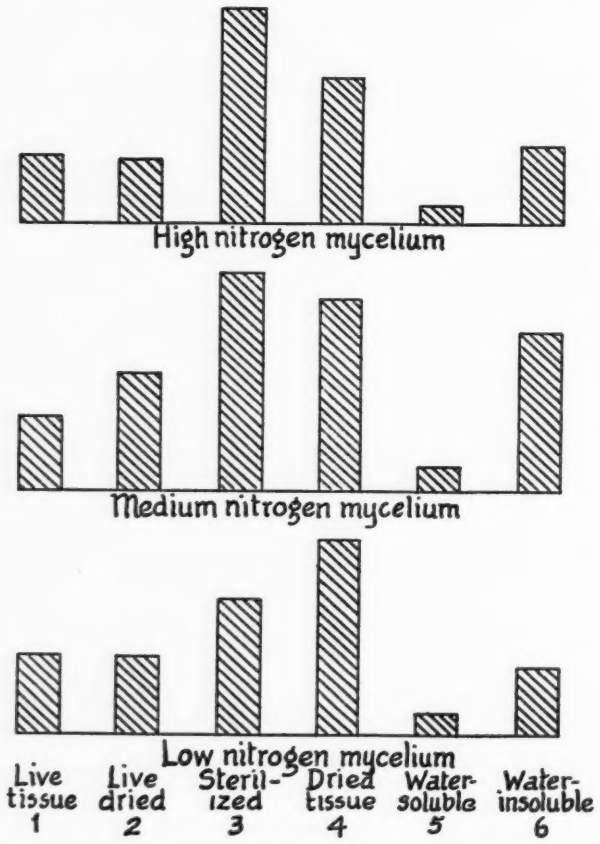


FIG. 1. RELATIVE BACTERIAL COUNTS FROM THE TISSUE OF ASPERGILLUS ORYZAE AFTER 40 DAYS IN THE SOIL

1. Fungous tissue incorporated in the soil in the living state.
2. Same as no. 1, but at the end of 20 days the entire soil mass was spread out and air-dried for 48 hours.
3. Sterilized at 15 pounds steam pressure for 45 minutes before being placed in the soil.
4. Fungous tissue dried at 65°C. before being placed in the soil.
5. Water-soluble fraction of no. 4.
6. Water-insoluble fraction of no. 4.

The number of bacteria ranged from 14.4 millions per gram of soil in the control to as high as 271 millions per gram where the mycelial tissue was sterilized with steam before its addition to the soil. The data in table 8 indicate that the number of bacteria are in direct relation to the amount of energy material suitable for their growth. Waksman and Starkey (121) have shown that glucose increases the number of bacteria in the soil and that cellulosic materials increase the amount of fungous tissue produced. The lowest bacterial count for any treatment was that for wheat straw, a substance low in soluble material but high in cellulosic energy material. In this case however, there was a very heavy mold growth in the soil. The dry mycelial tissue, on the other hand, gave high counts because of its high content of water-soluble energy material in the forms of the simpler carbohydrates, proteins, and amino acids. When the tissue was sterilized, the count was still higher because of the hydrolysis of some of the more complex substances. Living tissue gave a lower count than the dead tissue, there being less immediately available energy substance. The number of bacteria produced from the dry mycelium is approximately equal to the sum of those produced from its fractions. Figure 1 shows the relative number of bacteria produced from the various tissues of *Aspergillus oryzae*.

The amount of fungous material produced for each treatment could not be measured, but observation indicated heavy mold growth on all treatments where cellulosic or insoluble materials were added. The controls and the water-soluble fractions gave no noticeable fungous growth. The live tissue continued its growth from the energy in its own system but the dead tissue was immediately attacked by the saprophytic soil fungi and the entire soil was filled with their hyphae. There is no particular correlation between the number of bacteria and the amount of fungous hyphae produced, each developing independently of the other but depending upon the amount and kind of energy materials present. This is directly in line with the work of Fleming (39) who found that the development of bacteria and fungi in a soil depends upon the presence of organic matter, and that there is no evidence of a depressing effect of one upon the other, but that the relative abundance of the two groups depends upon their relative ability to utilize the energy materials present.

Accumulation of nitrate nitrogen

After intervals of 20, 40, and 80 days the accumulation of nitrate nitrogen from fungous tissue was determined. These data together with the percentages of the availability of the fungous nitrogen are given in table 9.

It is apparent that the more nitrogen added per unit of material the higher the availability of the nitrogen. This results from the fact that a given amount of energy material requires a certain amount of nitrogen for its decomposition, and any nitrogen in excess of this amount is liberated as nitrate. The figures show that from 200 mgm. of the dry tissue of *Aspergillus oryzae* in 100 gm. of

soil, from 4 to 5.2 mgm. of nitrogen remains unliberated as nitrate in 80 days. The amount of nitrogen not liberated in this way is fairly constant for a given amount of tissue during any period regardless of its nitrogen content, and roughly represents the amount of nitrogen used by soil microorganisms. If then the material contains less than this amount of nitrogen, none is liberated,

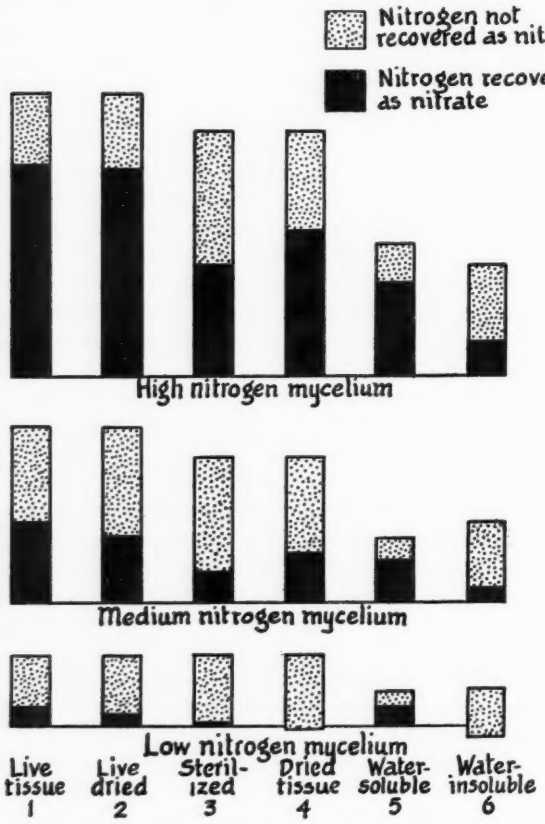


FIG. 2. RELATIVE AVAILABILITY OF THE NITROGEN IN THE TISSUE OF ASPERGILLUS ORYZAE AFTER 80 DAYS IN THE SOIL

1. Fungous tissue incorporated in the soil in the living state.
2. Same as no. 1, but at the end of 20 days the entire soil mass was spread out and air-dried for 48 hours.
3. Sterilized at 15 pounds steam pressure for 45 minutes before being placed in the soil.
4. Fungous tissue dried at 65°C. before being placed in the soil.
5. Water-soluble fraction of no. 4.
6. Water-insoluble fraction of no. 4.

but if it contains more than this amount, nitrate nitrogen is formed to the extent of the excess.

The nitrogen in living fungous tissue placed in the soil in the absence of other energy material is even more available than that in dead tissue. Drying at the end of 20 days tends to lessen the amount of nitrogen made available as nitrate. Observations indicate that in the living state fungous growth is continued and the energy for that growth in the absence of other energy material is derived from its own tissue through its autolysis. Dox (29, 30) and Dox and Maynard (32) found that when the energy material is exhausted from a culture medium, the organisms begin to autolize, using the energy from their own systems and liberating part of the nitrogen which they contain. The dry and sterilized tissues, both of which are dead, have a lower nitrogen availability than the living tissue. This is perhaps due to the heavy growth of saprophytic fungi on these tissues, although this is not true of the living material.

The water-soluble material from the fungous tissue has a higher nitrogen availability than either the water-insoluble or the total and its availability reaches its maximum in 20 days with no increase up to 80 days. This rapid nitrification is in accord with the findings of Whiting and Richmond (124), working with the parts of the sweet clover plant, that the roots, which contain large amounts of water-soluble material, nitrify much more rapidly at first than the leaves or stems. The water-soluble fungous nitrogen gives a high availability due largely to low cellulosic energy material and low fungous growth. The algebraic sum of the nitrogen not liberated from the water-soluble and water-insoluble fractions for any given period approximate that not liberated from an equal amount of the total tissue, and in every case the high energy residue has the low availability which is most marked in the low nitrogen tissue.

There is very little correlation of the availability of nitrogen with the number of bacteria but rather with the presence of energy material and more particularly with fungous growth. When the energy material is high and of such a nature as to produce a heavy fungous growth, the nitrogen availability is low. This is true in every case under observation in this work. It seems logical to believe that cellulosic energy materials in the soil cause the growth of saprophytic fungi and that these organisms use nitrogen in proportion to their growth or in proportion to the energy materials used, liberating only the nitrogen in excess of that necessary to balance this energy factor.

Figure 2 shows graphically the availability of the nitrogen in the tissue of *Aspergillus oryzae* and also that part of the nitrogen held insoluble after 80 days in the soil because of the energy factor.

EVOLUTION OF CARBON DIOXIDE AND ACCUMULATION OF NITRATE NITROGEN

It has already been shown that the ratio of carbon to nitrogen in the tissue is an important factor in the mineralization of fungous nitrogen, but there are

indications that it is not the only factor operating in this process. In order to study the relation of carbon to the formation of nitrates from fungous tissue and other sources of organic nitrogen, a second series of nitrification tests were carried out in flasks in the following manner: 600-gm. portions (dry weight) of a sandy mixture, made up of 25 per cent Miami silt loam and 75 per cent sand, were placed in a 750-cc. Erlenmeyer flask. The fungous tissues and other substances in amounts equivalent to 60 mgm. of nitrogen, or the water-soluble or -insoluble fraction thereof, were mixed with the soil. The moisture

TABLE 10

Ammonia and nitrate nitrogen from the decomposition of fungous tissue and other materials after 10 days' incubation

100 p.p.m. of nitrogen added

MATERIAL	AMMONIA NITROGEN	NITRATE NITROGEN
	p.p.m.	*p.p.m.*
Control	8.7	6.1
Ammonium sulfate	11.4	96.6
Blood meal	10.9	63.4
Cottonseed meal	9.8	47.0
Alfalfa hay	11.4	10.1
Timothy hay	9.3	Trace
Straw	8.7	Trace
Saccharomyces cerevisiae	11.4	66.2
Secotium acuminatum Mont.	10.9	40.2
Marasmius oreades (stipes)	9.2	42.0
Marasmius oreades (pilei)	9.8	50.0
Lycoperdon pyriforme	9.8	34.0
Trichoderma lignorum	9.3	42.8
Coprinus sp. (stipes)	9.8	33.0
Coprinus sp. (pilei)	9.8	33.0
Pholiota adiposa	8.7	None
Clitocybe multiceps	9.3	24.1
Fomes igniarius	10.9	10.4
Polyphorus graveolens	9.8	Trace

content was adjusted to 9 per cent (by weight) which was the optimum for this sand and soil mixture. In addition to this amount of moisture, 2 gm. of water was added for each gram of the organic material. The soil mixture gave a slightly alkaline reaction. The flasks were connected with a carbon dioxide absorption apparatus, which will be described in a later paper. The carbon dioxide evolved was determined at intervals of 12 to 72 hours. The aspiration was continuous over the soil except while the titrations were being made. All soil cultures were incubated at room temperature.

The amounts of carbon evolved as carbon dioxide were determined for a

period of 26 days as were also the amounts of nitrate nitrogen accumulated at that time. Ammonia and nitrate determinations were also made on duplicate treatments after a 10-day period. These data are given in table 10.

TABLE 11

Production of carbon dioxide and accumulation of nitrate nitrogen from the decomposition of fungous tissue and other organic materials

10 mg. nitrogen added to 100 gm. soil

MATERIAL	AMOUNT ADDED		NITRATE NITROGEN AFTER 26 DAYS	NITROGEN NITRIFIED	CARBON EVOLVED AS CARBON DIOXIDE		CARBON NITROGEN RATIO OF MATERIAL
	Dry weight	Carbon			Total	Last 72 hours	
	mgm.	*mgm.*	*mgm.**	*per cent*	*mgm.**	*mgm.†*	
Organic fertilizers and green manures							
Ammonium sulfate	47.5	.....	9.35	93.5	5.9	0.05	....
Blood meal	67.8	31.7	6.75	67.5	19.7	0.50	3.2
Cottonseed meal	150.5	67.2	4.95	49.5	34.8	0.55	6.7
Alfalfa hay	387.5	169.3	1.65	16.5	94.5	2.02	16.9
Timothy hay	840.3	373.8	−1.05	None	126.7	5.60	37.4
Straw	961.5	427.0	−1.05	None	113.2	5.47	42.7
Fungous tissue							
Saccharomyces cerevisiae	104.8	46.0	5.95	59.5	31.5	0.17	4.6
Secotium acuminatum	129.6	53.5	3.62	36.2	30.1	0.45	5.3
Marasmius oreades (pilei)	140.8	59.6	4.25	42.5	38.3	0.47	6.0
Marasmius oreades (stipes)	157.0	65.6	3.85	38.5	41.7	0.55	6.6
Coprinus sp. (pilei)	155.8	65.6	3.05	30.5	29.4	0.55	6.6
Lycoperdon pyriforme	198.3	84.3	3.25	32.5	49.8	0.70	8.4
Trichoderma lignorum	195.0	86.5	3.95	39.5	57.1	0.72	8.6
Aspergillus oryzae (1927)	216.6	91.5	4.2	42.0	59.2	0.95	9.2
Coprinus sp. (stipes)	250.0	97.0	3.35	33.5	57.6	0.77	9.7
Aspergillus oryzae (1926, med. N)	261.2	107.3	3.55	35.5	68.4	0.90	10.7
Clitocybe multiceps	267.3	108.5	2.75	27.5	64.8	0.90	10.9
Pholiota adiposa	260.3	108.6	−0.40	None	65.7	1.75	10.9
Coprinus radians	440.5	181.5	0.55	5.5	69.4	2.05	18.2
Fomes igniarius	584.8	259.0	0.35	3.5	15.1	1.62	25.9
Polyporus graveolens	641.0	267.3	−0.93	None	35.9	5.12	26.7

− means less than the control.

* Total minus that of the control.

† Carbon evolved as carbon dioxide last 72-hour period.

Tables 11 and 12 show the evolution of carbon as carbon dioxide and the accumulation of nitrate nitrogen during a period of 26 days. These tables also show the amounts of dry matter, carbon, and nitrogen incorporated in 100 gm. of soil.

AMMONIA AND NITRATE ACCUMULATION

The nitrogen in the form of ammonia at the end of 10 days was much the same for all treatments, ranging from 9 to 11 p.p.m., which is near the minimum usually found in a soil. This low ammonia content indicates that in these cultures the use of nitrogen by soil microorganisms and the process of nitrate

TABLE 12

Production of carbon dioxide and accumulation of nitrate nitrogen from the decomposition of the water-soluble and -insoluble fractions of various materials

Solubility fractions in 100 gm. of soil

MATERIAL	AMOUNT ADDED		NITRATE NITROGEN AFTER 26 DAYS	NITROGEN NITRIFIED	CARBON EVOLVED AS CARBON DIOXIDE		CARBON-NITROGEN RATIO OF MATERIAL
	Carbon	Nitrogen			Total	Last 72 hours	
	mgm.	*mgm.*	*mgm.**	*per cent*	*mgm.**	*mgm.†*	
Water-soluble fraction							
Aspergillus oryzae (1926, med. N)	14.8	4.52	2.65	54.2	14.4	0.22	3.3
Trichoderma lignorum	38.3	6.78	3.50	51.6	24.3	0.15	5.6
Lycoperdon pyriforme	34.2	5.66	2.45	43.6	22.8	0.10	6.1
Clitocybe multiceps	57.2	8.42	3.95	46.9	30.2	0.35	6.8
Cottonseed meal	10.7	1.11	0.45	40.3	8.75	0.33	9.6
Alfalfa hay	50.0	4.07	1.15	28.3	29.5	0.37	12.3
Water-insoluble fraction							
Cottonseed meal	56.5	8.89	4.5	50.9	29.8	0.28	6.3
Lycoperdon pyriforme	60.2	4.38	0.92	21.0	29.4	1.79	11.5
Trichoderma lignorum	48.2	3.22	0.80	24.9	28.9	2.02	14.9
Aspergillus oryzae (1926, med. N)	92.5	5.48	1.45	26.4	61.7	2.46	17.0
Alfalfa hay	119.3	5.93	0.56	9.6	59.8	2.00	20.1
Clitocybe multiceps	51.3	1.58	−0.71	None	33.7	9.06	32.4
Alkali-insoluble fraction							
Lycoperdon pyriforme	35.8	3.4	1.27	37.6	22.9	1.03	10.5
Clitocybe multiceps	40.3	1.6	−0.29	None	26.9	4.37	25.2

− means less than the control.

* Total minus that of the control.

† Carbon evolved as carbon dioxide during the last 72-hour period.

formation were going on as rapidly as the process of ammonification and thus preventing any accumulation of ammonia. The figures for nitrate nitrogen at 10 days show this very clearly. In the case of ammonium sulfate 90 per cent of the nitrogen added had gone over to nitrate and the nitrogen from the organic sources to the extent of 25 to 60 per cent. These data show that the slow accumulation of nitrate nitrogen is not due primarily so much to a

retarded nitrate formation as to a slow liberation of ammonia not used by microorganisms.

At 26 days the amounts of nitrate nitrogen had increased approximately 10 p.p.m. over those present at 10 days. A few of the low nitrogen fungous tissues gave little nitrification but most of them showed a mineralization of from 30 to 60 per cent of their nitrogen content, which was as good as or better than that of the other organic nitrogen carriers used. Straw and timothy hay gave a depression of nitrate nitrogen. When the materials were separated into their water-solubility fractions the water-soluble fraction always gave a greater and the water-insoluble fraction a lesser percentage of nitrate accumulation than the total, and in each case the sum of the nitrate nitrogen obtained from the two fractions approximate that from the total material.

THE RELATION OF CARBONACEOUS ORGANIC MATTER TO NITRATE ACCUMULATION

Amount of Carbon

When ammonium sulfate is introduced into a soil it is usually rather readily changed to nitrate, but if there is an available energy material present, less nitrogen will appear as nitrate in a given time. In this same way the carbon combined with organic nitrogen is a very definite factor in the mineralization of the nitrogen. The carbon-nitrogen ratio is an expression of the relative amounts of carbon and nitrogen in a substance and has often been used as an index of the availability of the nitrogen in an organic material. Batham (11), studying the mineralization of the nitrogen in pure organic compounds, found that the nitrate liberated is inversely proportional to the ratio of carbon to nitrogen in the substance. Concerning the relation of this ratio to the mineralization of organic nitrogen, Whiting (123) says: "With a high carbon content and a low nitrogen content nitrates may be produced slowly or not at all or they may be produced rapidly provided the carbon is not in a resistant state and the nitrogen of average to high content." This would indicate that not only the ratio of carbon to nitrogen but also the availability of the energy material is a factor in the mineralization of organic nitrogen. Tables 11 and 12 are arranged with the ratios of carbon to nitrogen in ascending order for the various groups of materials. These data show an inverse relationship between the carbon-nitrogen ratio and the mineralization process. A ratio higher than 11 or 12 to 1 gives a mineralization for 26 days of less than 30 per cent of the organic nitrogen. A lower ratio gives a somewhat higher nitrate accumulation. This relation seems to hold true with the fungous and other organic materials as well as with their water-solubility fractions. The ratio of carbon to nitrogen is not, however, truly inversely proportional to the nitrate accumulation, but rather a ratio of 12 to 1 seems to be about the dividing line between fair and little nitrate accumulation for a period of 26 days. This is shown by the fact that in 26 days, 35.5 per cent of the nitrogen was liberated from the tissue of *Aspergillus oryzae* with a carbon-nitrogen ratio of

10.7, 36.2 per cent from the tissue of *Secotium acuminatum* with a ratio of 5.3, and 30.5 per cent from that of a *Coprinus* sp. with a ratio of 6.6. These figures indicate that there are other factors operating besides that of the ratio of carbon to nitrogen.

The rate and amount of carbon evolved as carbon dioxide have been taken as a measure of biological activities in the soil and attempts have been made to correlate this with the mineralization process. The figures in table 11 for the amounts of carbon evolved as carbon dioxide from the various substances and fungous tissues indicate an inverse relation between this carbon and the amounts of nitrogen changed to nitrate, or a parallel with the ratio of carbon to nitrogen. This is true only in a general way and there are many exceptions

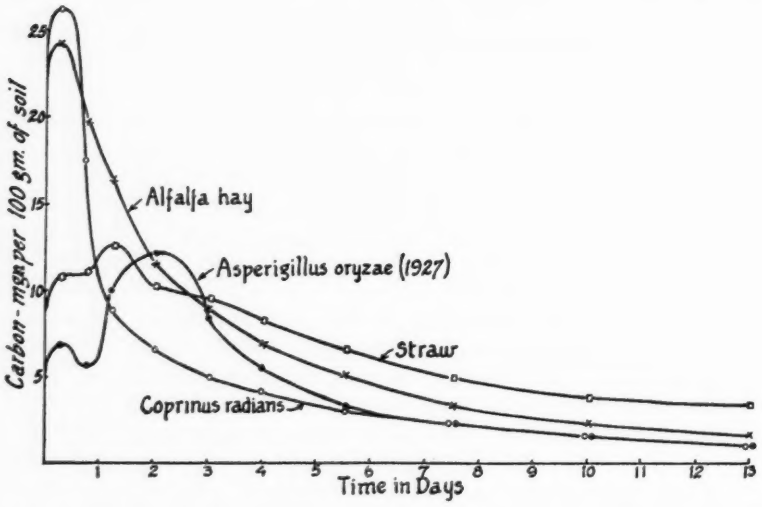


FIG. 3. RATE OF EVOLUTION OF CARBON DIOXIDE FROM VARYING AMOUNTS (10 MGM NITROGEN) OF DECOMPOSING ORGANIC MATTER IN MOIST SOIL

and some evidence to support the opposite view, especially in the case of a low nitrogen accumulation or of a depression. In the case of a depression, a lack of available nitrogen may cause retarded decomposition. In addition to the quantity of carbonaceous material present and the amount of carbon evolved as carbon dioxide for a given unit of nitrogen, there is evidence that the kind of carbonaceous material is also a factor in the mineralization process.

Kind of carbonaceous material

When organic matter decomposes in a soil a large amount of the carbon is evolved during the first few days. Figure 3 shows typical evolution curves for four of the materials used. All of these materials show the rapid develop-

ment of an early peak at about 12 hours with a more or less distinct second peak coming at from one to three days and then a gradual dropping off toward the control. With straw and the tissue of *Aspergillus oryzae* the second peak

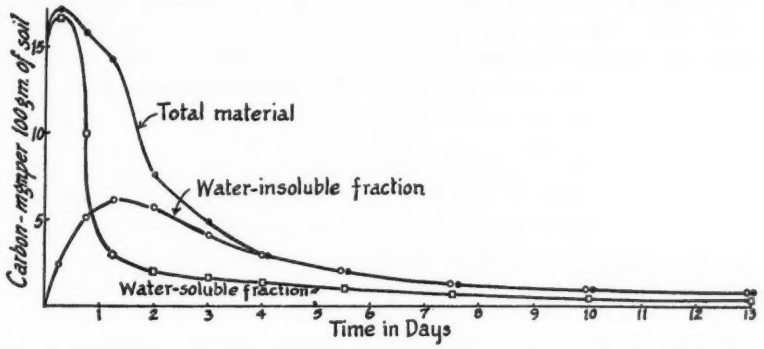


FIG. 4. EVOLUTION OF CARBON DIOXIDE FROM AN AMOUNT OF THE TISSUE OF LYCOPERDON PYRIFORME CARRYING 10 MGM. OF NITROGEN OR ITS WATER-SOLUBILITY FRACTIONS, WHEN DECOMPOSING IN MOIST SOIL

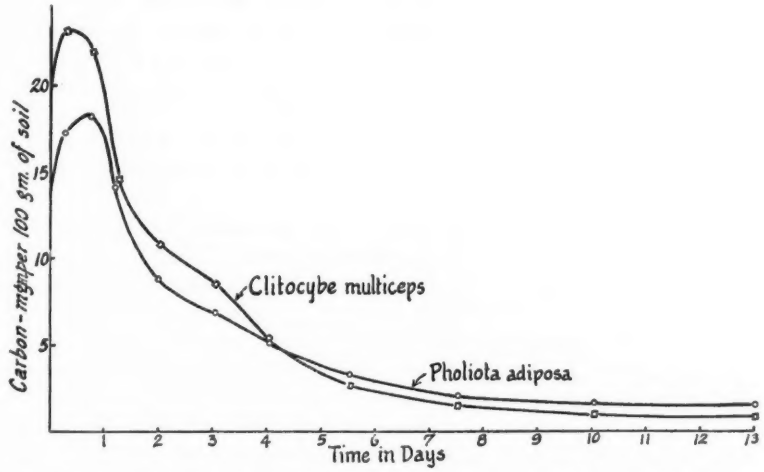


FIG. 5. EVOLUTION OF CARBON DIOXIDE FROM AMOUNTS OF TISSUE OF CLITOCYBE MULTICEPS AND PHOLIOTA ADIPOSA CARRYING 10 MGM. OF NITROGEN, WHEN DECOMPOSING IN MOIST SOIL

is well developed and the first one not so high, but with alfalfa and the tissue of *Coprinus radians* the first peak is high and well developed and the second one rather indistinct. The shape of these curves is indicative of the activities of soil microorganisms during a particular time interval and their height, the

intensity of this activity. The varying shapes and the double peaks indicate that the chemical composition or the chemical nature especially of the carbonaceous material is also variable. When the tissue of *Lycoperdon pyriforme* was separated into its fractions, each gave a distinctly different carbon evolution curve. Figure 4 shows that the water-soluble fraction gives an early peak with a rapid drop approaching the control, whereas the water-insoluble fraction gives a curve with a late peak and a slower decline toward the control. The sum of the carbon evolved from the two fractions gives a curve approximating that of the total.

The height of the evolution curves for carbon dioxide at the end of 26 days varies with the substances in question and is roughly in proportion to the ratio of carbon to nitrogen when a given amount of nitrogen is considered. Tables 11 and 12 show the amounts of carbon evolved as carbon dioxide during the last 72-hour period from an amount of material carrying 10 mgm. of nitrogen. It appears that the greater the amount of carbon evolved at this time the less the amount of nitrogen found as nitrate, and when this amount reaches approximately 1.7 to 2.0 mgm. of carbon for this period there is little or no nitrate accumulation. Without doubt the kind of energy material makes this difference. In table 11 in the cases of *Clitocybe multiceps* and *Pholiota adiposa*, the nitrogen contents of the tissues, the carbon-nitrogen ratios, and the total amounts of carbon evolved as carbon dioxide for 26 days are approximately the same, but from the former 27.5 per cent of its nitrogen is liberated as nitrate whereas from the latter there is a depression below the control. Figure 5 shows that the evolution curves are different, the former being high at the beginning and low at the end while the latter is just the reverse giving an evolution during the last 72 hours of 1.75 mgm. of carbon as against 0.90 mgm. for the former.

Thus it appears that the structure of the carbonaceous energy material governs somewhat the type of decomposition curves and at the same time the amount of nitrogen liberated as nitrate. If the energy materials are of simple nature their decomposition is rapid and their effect soon lost, but if they are of cellulosic form the decomposition is much slower and the action of the soil microorganisms is continued much longer with a corresponding inhibition in the accumulation of nitrate nitrogen. Allison (6) and Doryland (26) both felt that the energy-nitrogen relations are more important than the carbon-nitrogen relations as such, in that the rate of decomposition of the energy material depends much on its form and availability to microorganisms. Wilson and Wilson (126) have shown that with the same quantities of corn and sorghum roots in the soil, the depression of nitrate is different. In the early stages of decomposition the sorghum roots evolve more carbon dioxide than the corn roots and at the same time depress the nitrates to a greater extent. This they attribute to the greater amount of soluble energy material in the sorghum roots causing a greater biological activity. If the process is carried on longer than 30 days the reverse is true, more carbon dioxide is evolved from

the corn roots with a greater depression of nitrates than with the sorghum roots.

Type of organism

The form or kind of energy materials determines to a great extent the kind of organisms predominating in the decomposition. If these materials are

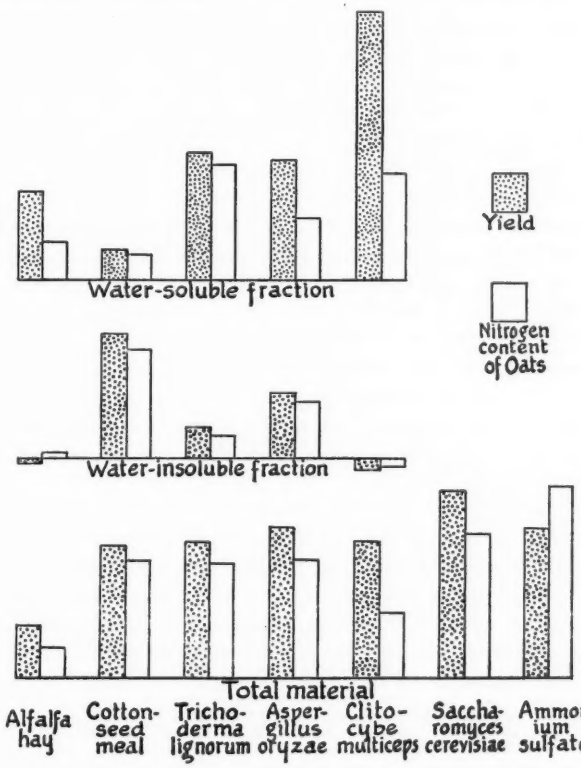


FIG. 6. RELATIVE YIELDS AND AMOUNTS OF NITROGEN ASSIMILATED BY OATS FROM FUNGOUS TISSUE AND OTHER NITROGEN CARRIERS (400 MGM. OF NITROGEN OR THE SOLUBILITY FRACTIONS WERE USED IN EACH CASE)

simple the decomposition will be largely bacterial and quickly completed, whereas if they are cellulosic the action will be largely fungous and of longer duration. The curve for the water-soluble fraction in figure 4 is typical for that of bacteria and that for the water-insoluble fraction for the fungi. These facts correlate with the observations of the fungous growth and bacterial counts as previously noted. Bacterial decomposition in the soil is very

complete and most of the carbon may be accounted for as carbon dioxide. The fungi, on the other hand, are more efficient in their use of carbon, for it has been shown (48) that as much as 40 per cent of the carbon used by fungi is found in their mycelial tissue. Neller (77) found in the decomposition of alfalfa meal, that fungi gave a greater evolution of carbon dioxide than bacteria, but that the latter showed a higher ammonia accumulation. This indicates that the bacteria attack the simpler carbonaceous compounds, build up very little protoplasm, and liberate much of the nitrogen as ammonia, whereas the fungi attack the cellulosic portion of the alfalfa meal with the production of much fungous tissue in which they use practically all of the nitrogen liberated in the decomposition process especially if the ratio of carbon to nitrogen is greater than 12 to 1.

On the whole it appears that the mineralization of organic nitrogen depends not only on the amount of carbonaceous or energy material with it, but also on the form and availability of this material and the resultant microflora. A given amount of energy material when used by soil organisms requires a given amount of nitrogen for its decomposition and this nitrogen is built up into the organic form in the microbial substance. Any nitrogen in excess of this amount is liberated in the mineral form. If the energy material is of a simple soluble nature, the decomposition is largely bacterial with a rapid evolution of the carbon as carbon dioxide. In this case a small amount of microbial protoplasm is formed with the result that a large amount of organic nitrogen is quickly changed to the inorganic form. If on the other hand the same amount of energy material is in a cellulosic form the decomposition is largely by fungi with a slower use of energy material extending over a longer period of time. These conditions lead to the formation of larger quantities of microbial substance than with the simpler energy materials and the use of greater quantities of nitrogen resulting in a lower nitrate accumulation. At the same time the effect of the energy extends over a longer period, maintaining the organisms in the living state and preventing the liberation of their nitrogen through autolysis or decomposition.

GROWTH OF OATS FROM FUNGOUS NITROGEN

In the previous work it has been found that the nitrogen in fungous tissue is readily changed to the mineral form in the soil. A series of plant cultures was arranged to test the ability of higher plants to use this nitrogen. Oats were used and the soil was the same as in the previous experiment. Here 10 kgm. of soil were used in a 2-gallon pot and 1 gm. of mono-potassium phosphate was added to each. The fungous tissues and other organic nitrogen carriers were prepared in the same way as described above. The total tissue was added in such an amount that it represented 400 mgm. of nitrogen per pot, and the water-soluble and water-insoluble portions carried the fractions of that amount that were soluble or insoluble in water. The oats were thinned to 12 plants per pot and watered with distilled water. The length of the growing period

was approximately 8 weeks. The tops only were harvested just before the heading stage, dried, ground, and the total nitrogen determined. Table 13 shows the results of the oat culture study including the yields and nitrogen recovery in the crop. The figures given are the averages for duplicate cultures. Plate 1 shows the oats at the time of harvest.

TABLE 13

The use by oats of nitrogen from decomposing fungous tissue and other nitrogen carriers

MATERIAL	MATERIAL ADDED PER POT			DRY WEIGHT, OATS PER POT	NITROGEN CONTENT OF OATS		NITROGEN RECOVERED IN OATS
	Total	Carbon	Nitrogen				
	gm.	*mgm.*	*mgm.*	*gm.*	*per cent*	*mgm.*	*per cent*
Controls	.....	.....	.....	7.35	1.17	86.0	.....
Ammonium sulfate	1.90	.....	400	12.5	2.85	356.2	67.5
Alfalfa hay, total	15.95	6,767	400	9.2	1.41	129.7	10.9
Alfalfa hay, water-soluble	.....	2,000	162.7	10.45	1.33	139.0	32.5
Alfalfa hay, water-insoluble	.....	4,767	237.3	7.15	1.33	95.1	3.9
Cottonseed meal, total	6.024	2,687	400	12.05	2.09	252.4	41.6
Cottonseed meal, water-soluble	.....	427	44.7	8.45	1.44	121.7	80.0
Cottonseed meal, water-insoluble	.....	2,260	355.3	12.1	1.96	237.2	42.3
Trichoderma lignorum, total	7.80	3,460	400	12.1	2.04	246.8	40.2
Trichoderma lignorum, water-soluble	.....	1,533	271.3	11.85	2.11	249.9	60.4
Trichoderma lignorum, water-insoluble	.....	1,927	128.7	8.4	1.40	117.6	24.8
Aspergillus oryzae (1927), total	8.7333	3,660	400	12.65	1.84	232.7	36.7
Aspergillus oryzae (1927), water-soluble	.....	390.4	120.5	11.55	1.49	172.1	71.4
Aspergillus oryzae (1927), water-insoluble	.....	3,269.6	279.5	9.7	1.71	165.9	28.6
Clitocybe multiceps, total	10.70	4,340	400	12.0	1.48	177.6	22.9
Clitocybe multiceps, water-soluble	.....	2,287	336.7	16.8	1.41	236.9	44.8
Clitocybe multiceps, water-insoluble	.....	2,053	63.3	6.95	1.22	84.8	−1.9
Saccharomyces cerevisiae, total	4.20	1,840	400	13.85	2.08	288.1	50.5

The water-soluble nitrogen has the highest availability of any fraction of the nitrogen. The range is from 40 to 70 per cent and is in accord with earlier experiments reported in this work. The high availability of the water soluble nitrogen is largely due to three factors: first, low amount of energy material or low carbon-nitrogen ratio; second, carbonaceous material which is readily decomposed and largely by bacterial action; and third, simple form of nitrogen.

Figures already given show that over half of the nitrogen in the water-soluble fraction is in the free amino form. This, together with its high dializability, indicates that the water-soluble nitrogen is very simple and easily decomposed. In such material the energy carbon is quickly evolved as carbon dioxide and little bacterial protoplasm is built up, thus liberating much of the organic nitrogen in the mineral form.

TABLE 14

A comparison of the availability of nitrogen in fungous tissue and other nitrogen carriers as measured by nitrification and oat culture experiments

MATERIAL	AVAILABILITY OF NITROGEN	
	In nitrification experiment	In oat culture experiment
	per cent	*per cent*
Ammonium sulfate	93.5	67.5
Alfalfa hay, total	16.5	10.9
Alfalfa hay, water-soluble	28.3	32.5
Alfalfa hay, water-insoluble	9.6	3.9
Cottonseed meal, total	49.5	41.6
Cottonseed meal, water-soluble	40.3	80.0
Cottonseed meal, water-insoluble	50.9	42.4
Trichoderma lignorum, total	39.5	40.2
Trichoderma lignorum, water-soluble	51.6	60.4
Trichoderma lignorum, water-insoluble	24.9	24.8
Aspergillus oryzae (1927), total	42.0	36.7
Aspergillus oryzae (1927), water-soluble	....	71.4
Aspergillus oryzae (1927), water-insoluble	....	28.6
Clitocybe multiceps, total	27.5	22.9
Clitocybe multiceps, water-soluble	46.9	44.8
Clitocybe multiceps, water-insoluble	−44.8	−1.9
Saccharomyces cerevisiae, total	59.5	50.5

− means less than the control.

The water-insoluble portion of all materials except cottonseed meal has a low availability, sometimes giving a depression of nitrate nitrogen. This is due not only to the high ratio of carbon to nitrogen but also to the kind of energy material present in the tissue. In this fraction the energy material is largely of the cellulosic type, which supports the growth of fungi to a much greater extent than bacteria. The fungi have a greater efficiency in the use of carbon and thus build up more carbon and at the same time more nitrogen into organic form.

The percentage availability of the nitrogen in the total tissue usually falls between those of the water-soluble and water-insoluble fractions. The amount of nitrate produced in the decomposition of fungous tissue is much the same as that produced from other organic nitrogen carriers of a similar nitrogen content. The amount of nitrogen in most of the fungous tissue studied ranged from 4 to 7 per cent and the availability of this nitrogen compared favorably with that of cottonseed meal which had a nitrogen content of 6.64 per cent. The species produced on artificial liquid culture media had a slightly higher amount of available nitrogen than did the natural forms, perhaps because of their slightly greater solubility in water.

There was a very close parallel between the percentages of the nitrogen changed to nitrate in the nitrification work and the amount of nitrogen found in the tops of the oat plants. Table 14 gives these data, and figure 6 shows graphically the relative yields of oats and the amounts of nitrogen assimilated by the crop. With the exception of the ammonium sulfate, a part of which no doubt was used in base exchange, the water-soluble portion of cottonseed meal, and the water-insoluble portion of *Clitocybe multiceps*, the latter two carrying low amounts of nitrogen, the parallel is almost perfect. As a rule the nitrogen obtained by the oats was slightly lower than the percentage of nitrification obtained in previous studies. Although the growth period of the oats was longer than the nitrification period, the tops of the oats only were harvested which would help to account for the slightly lower assimilation of nitrogen by the oats.

SUMMARY

A study was made of the nature and availability of the nitrogen in fungous tissue and in various organic materials. Fungous tissue was collected from natural habitats and also grown on artificial liquid culture media. The fungous material was separated into various chemical fractions and a study made of the carbon and nitrogen in these fractions as well as in the total material. The decomposition and nitrification of the various fungous tissues were studied in relation to carbon dioxide evolution and nitrate accumulation and also the effect of these tissues on the growth of higher plants.

The results of this work may be summarized as follows:

1. The carbon content of all fungous tissue studied is rather constant, fluctuating for the most part only between 40 and 44 per cent. The nitrogen content of the fungi found growing in the fields and woods varies from 1.5 to over 7 per cent, and the majority of these forms contain more than 4 per cent of nitrogen on a dry basis. In mycelium produced on synthetic liquid media, the nitrogen varied from less than 2 per cent to more than 6 per cent for the same species. The energy nitrogen ratio of the substrate is the determining factor, both in the quantity of the mycelium produced and the amount of nitrogen it contains. As the available nitrogen in the substrate decreases, the nitrogen content of the mycelium decreases to about 2 or 3 per cent. At this point a further decrease in the amount of nitrogen in the substrate causes a decrease in the weight of the mycelial tissue, so that the nitrogen content of the tissue seldom falls below 2 per cent. The decrease in the amount of the fungous tissue produced begins when its carbon-nitrogen ratio reaches 10 or 12 to 1.

2. Fungous nitrogen is for the most part very simple. From 40 to 70 per cent of the nitrogen in the dry fungous tissue used in this work was soluble in water, and of this portion, 80 to 92 per cent was dializable through a collodion sack. From 80 to 85 per cent of the total nitrogen was soluble in 0.05 *N* sodium hydroxide solution in 60 per cent alcohol. From 40 to 65 per cent of the nitrogen in the water-soluble and alcohol-soluble fractions was free amino nitrogen. The alcohol-soluble fraction was more complex, being only about 10 to 15 per cent free amino nitrogen. No urea was found in the tissue tested.

3. Most fungous tissues decompose readily in moist soils. From 40 to 60 per cent of their carbon is liberated as carbon dioxide in 26 days. On decomposition the nitrogen which they contain is liberated as nitrate to the extent of from 30 to 42 per cent of the original amount during a period of 26 days. The balance is either not liberated or is again combined into a new fungous or bacterial substance. When there is no other energy material present, living fungous tissue liberates its own nitrogen by autolysis to even a greater extent than the dead tissue. The rate of mineralization of fungous nitrogen depends upon the amount and kind of energy materials present. When the energy material is simple the decomposition is largely bacterial with the liberation of large amounts of nitrate nitrogen, but when it is of a cellulosic nature, the action is to a great extent fungous with the liberation of little or no mineral nitrogen. The nitrogen in fungous tissue in the soil is as readily nitrified as, or even more rapidly nitrified than that of other organic materials of similar nitrogen content.

REFERENCES

(1) Abbott, E. V. 1923 The occurrence and action of fungi in soils. *Soil Sci.* 16: 207–216.

(2) Abbott, E. V. 1926 Taxonomic studies of soil fungi. *Iowa State Col. Jour. Sci.* 1: 15–36.

(3) Abbott, E. V. 1926 A study of microbiological activities in some Louisiana soils. La. Agr. Exp. Sta. Bul. 194.

(4) Adametz, L. 1886 Untersuchungen über die niederen Pilze der Ackerkrume. *Inaug. Diss. Leipzig: Centbl. Bakt.* (1) 1: 8–10 (1887).

(5) Albrecht, W. A. 1926 Nitrate accumulation in soil as influenced by tillage and straw mulch. *Jour. Amer. Soc. Agron.* 18: 841–853.

(6) Allison, F. E. 1927 Nitrate assimilation by soil microörganisms in relation to available energy supply. *Soil Sci.* 24: 79–93.

(7) Association of Official Agricultural Chemists. 1925 Official and tentative methods of analysis compiled by the committee on editing methods of analysis, ed. 2. Washington, D. C.

(8) Barthel, C., and Bengtsson, N. 1924 Action of stable manure in the decomposition of cellulose in tilled soil. *Soil Sci.* 18: 185–200.

(9) Barthel, C., and Bengtsson, N. 1926 Bidrag till Frågen om Stallgödselkävets nitrifikation i åkerjorden. Meddel. Centralanst. Försöksv. Jordbruksområdet [Sweden] 311.

(10) Barthel, C., and Bengtsson, N. 1927 Availability of the nitrogen in fungi and bacterial cells for nitrification and cellulose decomposition in the soil. *Proc. First Internatl. Congr. Soil Sci.* 2: 204–208.

(11) Batham, H. N. 1927 Nitrification in soils: II. *Soil Sci.* 24: 187–203.

(12) Bierema, S. 1909 Die Assimilation von Ammon-, Nitrat- und Amidstickstoff durch Mikroorganismen. *Centr. Bakt.* (2) 23: 672–726.

(13) Blair, A. W., and Prince, A. L. 1928 The influence of heavy applications of dry organic matter on crop yields and on the nitrate content of the soil. *Soil Sci.* 25: 281–287.

(14) Brach, H. 1912 Untersuchungen über den chemischen Aufbau des Chitins. *Biochem. Ztschr.* 38: 468–491.

(15) BRACONNOT, H. 1811 De la Fongine, ou analyse des champignons. *Jour. Phys., Chim.* etc. 73: 130. From Lafar, Technical Mycology, v. 2, London, 1911.

(16) CARPENTIER, C. A. G. 1921 Studier över Stallgödselns inverkan på cellulosans sönderdelning i åkerjord. (Studies on the effect of manure on cellulose decomposition.) Meddel. Centralanst. Försöksv. Jordbruksområdet. [Sweden] 218. *Abs. Bact.* 6: 182 (1922).

(17) CHAMBERS, C. O. 1916. The fixation of free nitrogen by certain fungi. *Plant World* 19: 175–194.

(18) CLARK, E. D., AND SCALES, F. M. 1916 Enzymes of a cellulose-destroying fungus from the soil, *Penicillium pinophilum*. *Jour. Biol. Chem.* 24: xxxi.

(19) COLEMAN, D. A. 1916 Environmental factors influencing the activity of soil fungi. *Soil Sci.* 2: 1–65.

(20) COLLISON, R. E., AND CONN, H. J. 1925 The effect of straw on plant growth. N. Y. Agr. Exp. Sta. Tech. Bul. 114.

(21) CONN, H. J. 1916 Relative importance of fungi and bacteria in soil. *Science* 44: 857–858.

(22) CONN, H. J. 1922 A microscopic method for demonstrating fungi and actinomycetes in soil. *Soil Sci.* 14: 149–151.

(23) DALE, E. 1912 On the fungi of the soil. *Ann. Mycol.* 10: 452–477.

(24) DALE, E. 1914 On the fungi of the soil. *Ann. Mycol.* 12: 33–62.

(25) DORE, W. H., AND MILLER, R. C. 1923 The digestion of wood by *Teredo navalis*. *Cal. Univ. Pub. Zool.* 22 (6): 383–400.

(26) DORYLAND, C. J. T. 1916 The influence of energy material upon the relation of soil microorganisms to soluble plant food. N. D. Agr. Exp. Sta. Bul. 116.

(27) DOX, A. W. 1909 The intracellular enzymes of lower fungi, especially those of *Penicillium camemberti*. *Jour. Biol. Chem.* 6: 461–467.

(28) DOX, A. W. 1912 Enzyme studies of lower fungi. *Plant World* 15: 40–43.

(29) DOX, A. W. 1913 Autolysis of mold cultures: II. Influence of exhaustion of the medium upon the rate of autolysis of *Aspergillus niger*. *Jour. Biol. Chem.* 16: 479–484.

(30) DOX, A. W. 1915 The soluble polysaccharides of lower fungi: III. The influence of autolysis on the mycodextran content of *Aspergillus niger*. *Jour. Biol. Chem.* 20: 83–85.

(31) DOX, A. W., AND GOLDEN, R. 1911 Phytase in lower fungi. *Jour. Biol. Chem.* 10: 183–186.

(32) DOX, A. W., AND MAYNARD, L. 1912 Autolysis of mold cultures. *Jour. Biol. Chem.* 12: 227–231.

(33) DOX, A. W., AND NEIDIG, R. E. 1911 Pentosans in lower fungi. *Jour. Biol. Chem.* 9: 267–269.

(34) DOX, A. W., AND NEIDIG, R. E. 1914 The soluble polysaccharides of lower fungi: I. Mycodextran, a new polysaccharide in *Penicillium expansum*. *Jour. Biol. Chem.* 18: 167–175.

(35) DOX, A. W., AND NEIDIG, R. E. 1914 The soluble polysaccharides of lower fungi. II. Mycogalactan, a new polysaccharide in *Aspergillus niger*. *Jour. Biol. Chem.* 19: 235–237.

(36) DOX, A. W., AND ROARK, JR., G. W. 1920 The utilization of α-methylglucoside by *Aspergillus niger*. *Jour. Biol. Chem.* 41: 475–481.

(37) DUGGAR, B. M., AND DAVIS, A. R. 1916 Studies in the physiology of the fungi: I. Nitrogen fixation. *Ann. Missouri Bot. Gard.* 3: 413–437.

(38) FALCK, R. 1923 Mykologische Untersuchungen und Berichte, v. 2, Geb. Cassel., p. 15.

(39) FLEMING, W. E. 1925 The relation of fungi to the numbers of bacteria in the soil. *Soil Sci.* 19: 301–307.

(40) FRÉMY, M. E. 1859 Recherches sur la composition chimique des tissus des végétaux. *Jour. Pharm. et Chim.* 336: 5–14.

(41) GILMAN, J. C., AND ABBOTT, E. V. 1927 A summary of the soil fungi. *Iowa State Col. Jour. Sci.* 1: 225–345.

(42) GODDARD, H. N. 1913 Can fungi living in agricultural soil assimilate free nitrogen? *Bot. Gaz.* 56: 249–305.

(43) HARPER, H. J. 1924 The determination of ammonia in soils. *Soil Sci.* 18: 409–418.

(44) HARPER, H. J. 1924 The accurate determination of nitrates in soils. *Indus. Engin. Chem.* 16: 180–183.

(45) HAWKINS, L. A. 1915 The utilization of certain pentoses and compounds of pentoses by *Glomerella cingulata*. *Amer. Jour. Bot.* 2: 375–388.

(46) HAWLEY, L. F., FLECK, L. C., AND RICHARDS, C. A. 1928 Effect of decay on the chemical composition of wood. *Indus. Engin. Chem.* 20: 504–507.

(47) HEUKELEKIAN, H. 1924 Decomposition of cellulose by the various groups of microorganisms of the soil. *Abs. Bact.* 8: 9.

(48) HEUKELEKIAN, H., AND WAKSMAN, S. A. 1925 Carbon and nitrogen transformations in the decomposition of cellulose by filamentous fungi. *Jour. Biol. Chem.* 66: 323–342.

(49) ISHIDA, M., AND TOLLENS, B. 1911 Uber die Bestimmung von Pentosan und Methyl-Pentosan in Getreide und in Holzpilzen. *Jour. Landw.* 59: 59–67.

(50) ITERSON, JR., C. VAN. 1904 Die Zersetzung von Cellulose durch aërobic Mikroorganismen. *Centbl. Bakt.* (2) 11: 689–698.

(51) IWANOFF, N. N. 1923 Über den harnstoffgehalt der Pilze. *Biochem. Ztschr.* 136: 1–8.

(52) IWANOFF, N. N. 1923 Über die bildung des harnstoffs in Pilzen. *Biochem. Ztschr.* 136: 9–19.

(53) IWANOFF, N. N. 1923 Über das Viscosin der Pilze. *Biochem. Ztschr.* 137: 320–330.

(54) IWANOFF, N. N. 1923 Über die Nature des Eiweiszstoffes der Pilze. *Biochem. Ztschr.* 137: 331–340.

(55) IWANOFF, N. N. 1924 Über die Ursache des verschiedenen Harnstoffgehalts in Pilzen. *Biochem. Ztschr.* 154: 391–398.

(56) IWANOFF, N. N. 1928 Über Harnstoff in Pilzen. *Biochem. Ztschr.* 192: 36–40.

(57) IWANOFF, N. N., AND TOSCHEWIKOWA, A. 1927 Über zwei Arten von Harnstoffbildung bei Champignons. *Biochem. Ztschr.* 181: 1–7.

(58) JENSEN, C. N. 1912 Fungous flora of the soil. N. Y. (Cornell) Agr. Exp. Sta. Bul. 315.

(59) KOTAKE, Y., AND SERA, Y. 1913 Über eine neue Glukosaminverbindung, zugleich ein Beitrag zur Konstitutionsfrage des Chitins. *Ztschr. Physiol. Chem.* 88: 56–72.

(60) KLEIN, G., EIGNER, A., AND MÜLLER, H. 1926 Nitratassimilation bei Schimmelpilzen. *Ztschr. Physiol. Chem.* 159: 201–234.

(61) KLOTZ, L. J. 1923 Studies in the physiology of the fungi. XVI. Some aspects of nitrogen metabolism in fungi. *Ann. Missouri Bot. Gard.* 10: 299–368.

(62) KOPELOFF, N. 1916 The inoculation and incubation of soil fungi. *Soil Sci.* 1: 381–403.

(63) KOSSOWICZ, A. 1914 Zur Frage der Assimilation des elementaren Stickstoffs durch Hefen und Schimmelpilze. *Biochem. Ztschr.* 64: 82–85.

(64) KRESS, O., ET AL. 1925 Control of decay in pulp and pulp wood. U. S. Dept. Agr. Bul. 1298.

(65) LAFAR, F. 1904–1907 Handbuch der Technischen Mykologie, v. 5. Jena.

(66) LIPMAN, C. B. 1911 Nitrogen fixation by yeasts and other fungi. *Jour. Biol. Chem.* 10: 169–182.

(67) LYON, T. L. 1926 Nitrates in the soil as influenced by the growth of plants. *Jour. Amer. Soc. Agron.* 18: 834–840.

(68) LYON, T. L., BIZZELL, J. A., AND WILSON, B. D. 1923 Depressive influence of certain higher plants on the accumulation of nitrates in soil. *Jour. Amer. Soc. Agron.* 15: 457–467.

(69) MCBETH, I. G., AND SCALES, F. M. 1913 The destruction of cellulose by bacteria and filamentous fungi. U. S. Dept. Agr. Bur. Plant Indus. Bul. 266.

(70) MCLEAN, H. C., AND WILSON, G. W. 1914 Ammonification studies with soil fungi. N. J. Agr. Exp. Sta. Bul. 270.

(71) MCLENNAN, E. 1928 The growth of fungi in soil. *Ann. Appl. Biol.* 15: 95–109.

(72) MARSHALL, JR., E. K. 1913 A rapid clinical method for the estimation of urea in urine. *Jour. Biol. Chem.* 14: 283–290.

(73) MAZÉ, P. 1902 Recherches sur les modes d'utilisation du carbone ternaire par les végétaux et les microbes. *Ann. Inst. Past.* 16: 346–378; 433–451.

(74) MURRAY, T. J. 1921 The effect of straw on the biological soil processes. *Soil Sci.* 12: 233–259.

(75) MÜTTERLEIN, C. 1913 Studien über die Zersetzung der Cellulose im Dünger und im Boden. Univ. Leipzig Diss.

(76) NEIDIG, R. E. 1913 Polyatomic alcohols as sources of carbon for lower fungi. *Jour. Biol. Chem.* 16: 143–145.

(77) NELLER, J. R. 1918 Studies on the correlation between the production of carbon dioxide and the accumulation of ammonia by soil organisms. *Soil Sci.* 5: 225–241.

(78) OUDEMANS, C. A. J. C., AND KONING, C. J. 1902 Prodrome d'une flore mycologique, obtenue par la culture sur gelatin preparée de la terre humeuse du spanderswoud près de Bussum. *Arch. Néerland. Sci. Exact. et Nat.* (2) 7: 266–298.

(79) PAINE, F. S. 1927 Studies of the fungous flora of virgin soils. *Mycologia.* 19: 248–267.

(80) PETERSON, W. H., FRED, E. B., AND SCHMIDT, E. G. 1922 The fermentation of pentoses by molds. *Jour. Biol. Chem.* 54: 19–34.

(81) PRATT, O. A. 1918 Soil fungi in relation to diseases of the Irish potato in southern Idaho. *Jour. Agr. Res.* 13: 73–100.

(82) PRINGSHEIM, H. 1910 Studien über die Spaltung racemischer Aminosaüren durch Pilze. *Ztschr. Physiol. Chem.* 65: 96–109.

(83) PRINGSHEIM, H., AND ZEMPLEN, G. 1909 Studien über die Polysaccharide spaltenden Fermente in Pilzpreszsäften. *Ztschr. Physiol. Chem.* 62: 367–385.

(84) PROSKURIAKOW, N. J. 1926 Über die Beteiligung des Chitins am Aufbau der Pilzzellwand. *Biochem. Ztschr.* 167: 68–76.

(85) RAHN, O. 1919 Die schädliche Wirkung der Strohdüngung und deren Verhütung. *Ztschr. Tech. Biol.* 7: 172–186.

(86) RATHBUN, A. E. 1918 The fungous flora of pine seed beds. *Phytopathology* 8: 469–483.

(87) REGE, R. D. 1927 Biochemical decomposition of cellulosic materials, with special reference to the action of fungi. *Ann. Appl. Biol.* 14: 1–44.

(88) REUTER, C. 1912 Beiträge sur Kenntnis der Stickstoffhaltigen Bestandteile der Pilze. *Ztschr. Physiol. Chem.* 78: 167–245.

(89) RUSSELL, E. J. 1923 The Microorganisms of the Soil, chap. 7 and 8. London.

(90) SCALES, F. M. 1914 The enzymes of *Aspergillus terricola*. *Jour. Biol. Chem.* 19: 459–472.

(91) SCALES, F. M. 1915 Some filamentous fungi tested for cellulose destroying power. *Bot. Ga.* 60: 149–153.

(92) SCHENKER, R. 1921 Zur Kenntnis der Lipase von *Aspergillus niger* (van Tiegh). *Biochem. Ztschr.* 120: 164–196.

(93) SCHMIDT, E. G., PETERSON, W. H., AND FRED, E. B. 1923 The destruction of pentosans by molds and other microörganisms. *Soil Sci.* 15: 479–488.

(94) SCHRENK, H. VON. 1900 Two diseases of red cedar caused by *Polyporus juniperinus* n. sp. and *Polyporus carneus* Nees. U. S. Dept. Agr. Veg. Physiol. and Path. Bul. 21.

(95) SCOTT, H. 1921 The influence of wheat straw on the accumulation of nitrates in the soil. *Jour. Amer Soc. Agron.* 13: 233–258.

(96) SIEBER, N. 1881 Beiträge zur Kenntniss der chemischen Zusammensetzung der Schimmelpilze. *Jour. Prakt. Chem.* (N. F.) 23: 412–421.

(97) SIEVERS, F. J., AND HOLTZ, H. F. 1926 The significance of nitrogen in soil organic matter relationships. Wash. Agr. Exp. Sta. Bul. 206.

(98) SKINNER, C. E. 1925 What organisms are responsible for the decomposition of cellulose in the soil? *Abs. Bact.* 9: 32.

(99) STARKEY, R. L. 1924 Some observations on the decomposition of organic matter in soils. *Soil Sci.* 17: 293–314.

(100) STARKEY, R. L. 1924 Evolution of carbon dioxide as an index of decompsotion of organic matter in the soil. *Abs. Bact.* 8: 9.

(101) STEINBERG, R. A. 1919 A study of some factors in the chemical stimulation of the growth of *Aspergillus niger*. *Amer. Jour. Bot.* 6: 330–372.

(102) TANRET, M. C. 1897 Recherches sur les champignons. *Bul. Soc. Chim. Paris* (3) 17: 921–927.

(103) THOM, C., AND LATHROP, E. C. 1925 Psilocybe as a fermenting agent in organic. debris. *Jour. Agr. Res.* 30: 625–628.

(104) VAN SLYKE, D. D. 1912 The quantitative determination of aliphatic amino groups. II. *Jour. Biol. Chem.* 12: 275–284.

(105) VAN SLYKE, D. D. 1913 The gasometric determination of aliphatic amino nitrogen in minute quantities. *Jour. Biol. Chem.* 16: 121–124.

(106) VILJOEN, J. A., AND FRED, E. B. 1924 The effect of different kinds of wood and wood pulp cellulose on plant growth. *Soil Sci.* 17: 199–211.

(107) VORBRODT, W. 1926 O przeróbce azotu w grzybni kropidlaka (*Aspergillus niger*). (Elaboration of nitrogen in the mycelium of *A. niger*.) *Bul. Internatl. Acad. Polonaise Sci. et Lett.* (*B*) *Sci. Nat.* (5/6B): 517–533. Abs. in *Biol. Abs.* 1 (11440): 1025 (1928).

(108) WAKSMAN, S. A. 1916 Soil fungi and their activities. *Soil Sci.* 2: 103–156.

(109) WAKSMAN, S. A. 1917 Is there any fungus flora of the soil? *Soil Sci.* 3: 565–589.

(110) WAKSMAN, S. A. 1918 Studies on proteolytic activities of soil microörganisms with special reference to fungi. *Jour. Bact.* 3: 475–492.

(111) WAKSMAN, S. A. 1918 Studies on the proteolytic enzymes of soil fungi and actinomycetes. *Jour. Bact.* 3: 509–530.

(112) WAKSMAN, S. A. 1922 The growth of fungi in the soil. *Soil Sci.* 14: 153–157.

(113) WAKSMAN, S. A. 1926 The origin and nature of the soil organic matter or soil "humus:" III. The nature of the substances contributing to the formation of humus. *Soil Sci.* 22: 323–333.

(114) WAKSMAN, S. A. 1926 On the origin and nature of the soil organic matter or soil "humus:" V. The rôle of microörganisms in the formation of "humus" in the soil. *Soil Sci.* 22: 421–436.

(115) WAKSMAN, S. A. 1927 Principles of Soil Microbiology. Baltimore.

(116) WAKSMAN, S. A., AND COOK, R. C. 1916 Incubation studies with soil fungi. *Soil Sci.* 1: 275–284.

(117) WAKSMAN, S. A., AND HEUKELEKIAN, O. 1924 Microbiological analysis of soil as an index of soil fertility: VIII. Decomposition of cellulose. *Soil Sci.* 17: 275–291.

(118) WAKSMAN, S. A., AND HEUKELEKIAN, H. 1926 Cellulose decomposition by various groups of soil microorganisms. *Actes de la IVeme Conf. Internatl. Pedologie* 3: 216–226.

(119) WAKSMAN, S. A., AND TENNEY, F. G. 1926 On the origin and nature of the soil organic matter or soil "humus:" IV. The decomposition of the various ingredients of straw and of alfalfa meal by mixed and pure cultures of microörganisms. *Soil Sci.* 22: 395–406.

(120) WAKSMAN, S. A., AND SKINNER, C. E. 1926 Microörganisms concerned in the decomposition of celluloses in the soil. *Jour. Bact.* 12: 57–84.

(121) WAKSMAN, S. A., AND STARKEY, R. L. 1924 Influence of organic matter upon the development of fungi, actinomycetes, and bacteria in the soil. *Soil Sci.* 17: 373–378.

(122) WHITE, J. W., AND HOLBEN, F. J. 1925 Perfection of chromic acid method for determining organic carbon. *Indus. Engin. Chem.* 17: 83–85.

(123) WHITING, A. L. 1926 Some important factors controlling the rate of nitrification of organic materials. *Jour. Amer. Soc. Agr.* 18: 854–876.

(124) WHITING, A. L., AND RICHMOND, T. E. 1927 The relative rates of nitrification of different parts of sweet clover plants. *Soil Sci.* 24: 31–37.

(125) WICHERS, J. L., AND TOLLENS, B. 1910 III. Über die Pentosane einiger Holzpilze. *Jour. Landw.* 58: 238–242.

(126) WILSON, B. D., AND WILSON, J. K. 1928 Relation of sorghum roots to certain biological processes. *Jour. Amer. Soc. Agron.* 20: 747–754.

(127) WINTERSTEIN, E. 1893 Zur Kenntniss der Pilzcellulose. *Ber. Deut. Bot. Gesell.* 11: 441–445.

(128) WINTERSTEIN, E. 1894 Zur Kenntniss der in den Membranen der Pilze enthaltenen Bestandtheile. *Ztschr. Physiol. Chem.* 19: 521–562.

(129) WINTERSTEIN, E. 1894 Ueber ein stickstoffhaltiges Spaltungsproduct der Pilzecellulose. *Ber. Deut. Chem. Gesell.* 27: 3113–3115.

(130) WINTERSTEIN, E. 1895 Zur Kenntniss der in den Membranen der Pilze enthaltenen Bestandtheile. *Ztschr. Physiol. Chem.* 21: 134–151.

(131) WINTERSTEIN, E. 1895 Ueber Pilzcellulose. *Ber. Deut. Bot. Gesell.* 13: 65–70.

(132) WINTERSTEIN, E., AND REUTER, C. 1912 Über die Stickstoffhaltigen Bestandteile der Pilze. *Centbl. Bakt.* (2) 34: 566–573.

(133) WINTERSTEIN, E., REUTER, C., AND KOROLEW, R. 1913 Über die Chemische Zusammensetzung einiger Pilze und über die bei der Autolyse derselben auftretenden Producte. *Landw. Vers. Sta.* 79/80: 541–562.

(134) YOSHIMURA, K., AND KANIA, M. 1913 Beträge zur Kenntnis der Stickstoffhaltigen Bestandteile des Pilzes *Cortinellus shiitake* P. Henn. *Ztschr. Physiol. Chem.* 86: 178–184.

(135) ZELLER, S. M. 1916 Studies in the physiology of the fungi. II. *Lenzites saepiaria*, Fries., with special reference to enzyme activity. *Ann. Missouri Bot. Gard.* 3: 439–512.

(136) ZELLNER, J. 1907 Chemie der höheren Pilze. Leipzig.

PLATE 1

FIG. 1. Comparative growth of oats from 400 mgm. of nitrogen from various sources.

FIG. 2. Comparative growth of oats from the water-soluble and water-insoluble nitrogen from alfalfa hay and cottonseed meal.

FIG. 3. Comparative growth of oats from the water-soluble and water-insoluble nitrogen from *Trichoderma lignorum* and *Aspergillus oryzae* (1927).

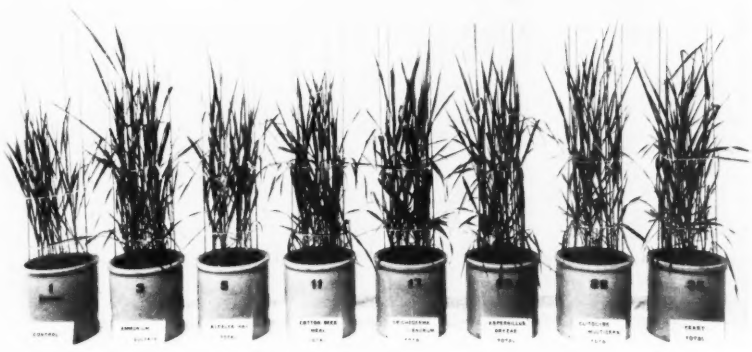


FIG. 1

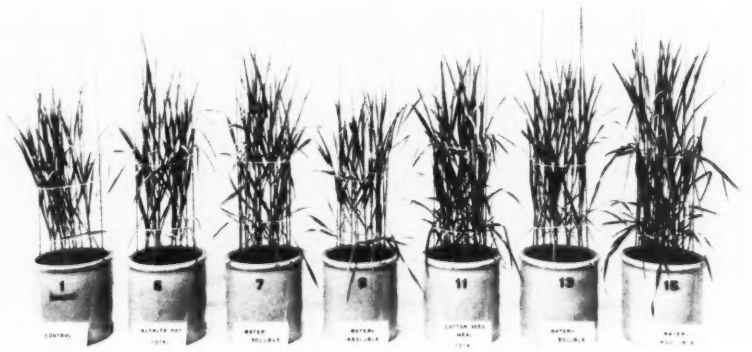


FIG. 2

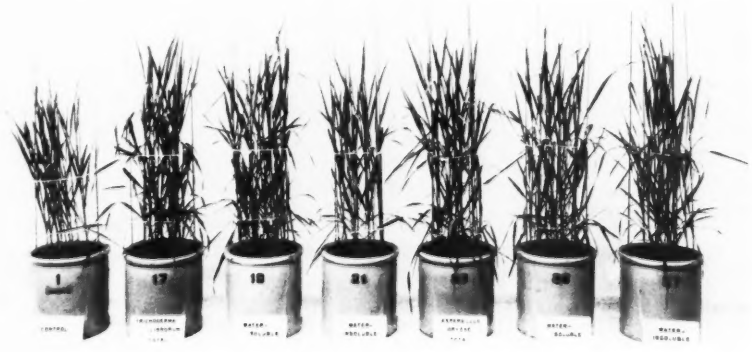


FIG. 3

THE INFLUENCE OF THE REPLACEABLE BASES ON THE SOIL SOLUTION FORMATION IN MINERALIZED SOILS

F. MENCHIKOWSKY AND S. RAVIKOVITCH

Palestine Zionist Executive Agricultural Experiment Station

Received for publication September 6, 1928

A considerable number of papers are devoted to the question of the formation and composition of the soil solution. In one of them, Whitney and Cameron (13) have reported that: "If every soil contains all the common rock forming minerals, every soil should give the same saturated solution."

The following investigations of the seasonal change in the soil solution composition (3, 11, 12) have shown, on the contrary, that the composition of the soil solution is in no way constant but that it changes in relation to the biochemical conditions of the soil and the growth of the plant.

On the basis of experiments carried out on leached soils, Burd (2) emphasized the importance of the biological processes for cation enrichment of the soil solution. On the grounds of these observations, Hoagland (8) defines the conditions of the soil solution formation in the following manner: "Normally a soil solution is in long measure a biologically controlled system; that is to say that nearly all the anion content of such a solution is of biological origin and equivalent quantities of cations must enter into solution with the anions."

The soil solution is composed of different parts. The salts of mineral origin which we detect in every soil, and chiefly in arid soils, may be dissolved at first if the soil contains a sufficient amount of water. The biochemical activity, on the other hand, as was pointed out in the foregoing, enriches the soil solution by anions and, in consequence of their action, also by cations taken from the insoluble portion of the soil. This source of the formation of the soil solution is very considerable, for the soils which have an important amount of organic matter are consequently characterized by their highly developed biological activity. As a source of the soluble parts of the soil solution we must take into account also the hydrolysis of the acid colloids of the soil, saturated with bases.

In the literature dealing with the question of soil zeolites some observations can be found about the conditions of the formation and decomposition of the zeolitic compounds with bases. Gedroiz (5) classifies the zeolites saturated with bases according to their stability in the following manner: Zeolites bound with CaO and MgO belong to the most durable compounds; those saturated with Na_2O and K_2O are less stable; the intermediate properties are shown by the zeolites saturated with H ion. These compounds of zeolites with bases are hydrolyzed by water with the appearance of free bases in the solution.

In the highly mineralized soils where organic matter is perhaps very insignificant, or where it is sometimes absent, the biochemical activity is consequently very low. The biological factor of the formation of the soil solution in those soils is therefore very limited. Although the influence of the slightly soluble minerals should not be important, the formation of the soil solution in such a case depends on the amount of easily soluble salts, but the chief factor here should be in the hydrolysis of the zeolitic compounds with bases.

The object of the experiments reported in this paper was to verify this view on the bases of some mineralized soils of Palestine.

SAMPLES OF SOIL FOR ANALYSIS

Three samples of soil from different parts of Palestine were chosen as material for study: 1. A sample of the heavy loamy soil from the coastal region in the

TABLE 1

The amount of organic matter in the soil samples

NAME OF LOCALITY	LAYER	PERCENTAGE OF DRY MATTER
	cm.	
Ben-Shemen	0– 25	0.81–0.78
Ben-Shemen	25– 50	0.61–0.59
Ben-Shemen	50– 75	0.54–0.52
Ben-Shemen	75–100	0.49–0.49
Djuania	0– 25	1.39–1.30
Djuania	25– 50	1.09–1.02
Djuania	50– 75	0.89–0.85
Djuania	75–100	0.65–0.65
Dagania	0– 25	1.22–1.19
Dagania	50– 75	0.56–0.53

vicinity of Ben-Shemen; 2. A sample of heavy loamy soil from the center of the Plain of Esdraelon south of Afule, from a place named Djuania; and 3. A sample of soil rich in lime from the Jordan Valley. The amount of organic matter in all these samples is insignificant, as is shown in table 1.

The samples employed for the investigation were taken from four layers one below the other and each 25 cm. deep. In order to establish the relation existing between the replaceable bases of the soil and the soil extracts, the replaceable bases were studied in all the aforementioned samples.

The properties of the local heavy loamy soil make the preparation of the soil solution very difficult. In this work the water extract method was used. The experiments were carried out with 15, 25, 50, and 100 parts of soil to 100 parts of water. The comparison of the figures of the different water extracts makes it possible to determine the compounds which existed in the soil solution as distinct from those formed by hydrolysis.

THE METHODS

Methods have been proposed for the estimation of the amount of replaceable bases in a soil. The oldest method proposed by Gedroiz is based on the replacing of the absorbed bases by a solution of NH_4Cl; the second method recently recommended by him is the replacing with N 0.05 HCl (4). These methods, however, can not be applied in the presence of $CaCO_3$ in the soils, because both N 0.05 HCl and NH_4Cl dissolve $CaCO_3$. For the same reason, the method proposed by Bobko-Askenasi (1), based on the treating with $BaCl_2$, can not be applied. The most suitable method for Palestine soils rich in $CaCO_3$ is that of Hissink (7), based on the replacing of the absorbed Ca by the solution of NaCl. Hissink found that the quantity of $CaCO_3$ dissolved in one liter of NaCl is constant and that by a second and third treatment of the same

TABLE 2

The amount of CaO extracted from the soil by the second liter of the normal NaCl solution*

LOCALITY	LAYER	CaO IN 200 CC. N NaCl SOLUTION
	cm.	*gm.*
Ben-Shemen	0– 25	0.0089
Ben-Shemen	25– 50	0.0093
Ben-Shemen	50– 75	0.0090
Ben-Shemen	75–100	0.0090
Djuania	0– 25	0.0101
Djuania	25– 50	0.0097
Djuania	50– 75	0.0101
Djuania	75–100	0.0096
Dagania	0– 25	0.0091
Dagania	25– 50	0.0090

* $CaCO_3$ in the same soils: Ben-Shemen, 15.5 per cent; Djuania, 17.5 per cent; Dagania, 40.1 per cent.

soil with the solution of NaCl the further half liters of the NaCl solution show the same amount of dissolved $CaCO_3$. The value of this method was confirmed by the determinations of the absorbed Ca in our soils. The constant amount of the dissolved $CaCO_3$ in each second liter of NaCl solution after the soil has been treated should be observed. The figures are shown in table 2.

The soil extracts were prepared from air-dry soil passed through the 2-mm. sieve and shaken with water for one hour. The liquid was filtered through the funnel without contact with the air. The paper for the filtration was washed several times with distilled water and was checked as to presence of soluble salts and traces of acid.

The following methods were used for the estimation of anions: Cl^- was determined by titration with 0.02 N $AgNO_3$; HCO_3^- with 0.1 N HCl in the presence of methyl orange; NO_3^- was estimated by the colorimetric method with phenol-sulfonic acid. Before the soil was tested, Cl^- was separated with

Ag_2SO_4. For $PO_4^{\equiv}$ the determination method of Deniges, modified by Shmuk and Kurilo (9) was employed. $SiO_3^{=}$ was also measured colorimetrically (10). The method is based on the color formed by ammonium molybdate. The several tests of the standard solution from K_2SiO_3 have shown that the boiling of the solution and the addition of the strong acid, lead to the partial coagulation of SiO_2. This method was therefore changed. To the solution was added diluted HNO_3 (d = 1.12, 1:4) and 5 per cent ammonium molybdate. The tested solution was not heated. The cations Ca, Mg, K, and Na were

TABLE 3

The amount of replaceable bases in the soils tested

ORIGIN OF A SAMPLE	MGM. EQUIVALENT IN 100 GM. DRY SOIL					PERCENTAGE OF A REPLACEABLE CATION TO THE TOTAL OF REPLACEABLE BASES IN 100 GM. DRY SOIL					
	Ca	Mg	K	Na	Total	Ca	Mg	K	Na	Ca + Mg	K + Na
The heavy loamy soil from Ben-Shemen											
cm.											
0– 25	41.9	17.2	0.85	6.73	66.68	62.8	25.8	1.3	10.1	88.6	11.4
25– 50	38.8	16.4	0.59	3.91	59.70	65.0	27.4	1.0	6.6	92.4	7.6
50– 75	36.4	15.5	0.36	7.62	59.88	60.8	25.8	0.6	12.8	86.6	13.4
75–100	33.5	18.5	0.26	11.97	64.23	52.2	28.8	0.4	18.6	81.0	19.0
The heavy loamy soil from Djuania											
cm.											
0– 25	33.1	19.9	1.27	3.78	58.05	57.0	34.3	2.2	6.5	91.3	8.7
25– 50	29.4	21.9	0.64	4.92	56.86	51.7	38.5	1.1	8.7	90.2	9.8
50– 75	25.9	22.5	0.41	5.04	53.85	48.1	41.8	0.7	9.4	89.9	10.1
75–100	24.1	22.7	0.35	7.64	54.79	44.0	41.4	0.6	14.0	85.4	14.6
Soil rich in lime from Jordan Valley (Dagania)											
cm.											
0 –25	26.6	5.47	0.41	1.50	33.98	78.3	16.1	1.2	4.4	94.4	5.6
25– 50	24.8	6.23	0.12	1.78	32.93	75.3	18.9	0.4	5.4	94.2	5.8
50– 75	21.5	7.57	....	2.62	31.69	67.8	23.9	...	8.3	91.7	8.3
75–100	18.3	10.31	....	2.34	30.95	59.1	33.3	...	7.6	92.4	7.6

measured by the common analytical method. All the calculations are made on dry matter.

REPLACEABLE BASES IN SOILS

The results of the study of the aforementioned soils concerning the bases absorbed by the colloidal part are reported in table 3. From the figures obtained it appears that these three soils possess a different quantity of replaceable bases. The heavy loamy soil of Ben-Shemen has the largest quantity

of replaceable bases, 64–67 mgm.—equivalents in 100 gm. of soil; the sample from the Plain of Esdrealon, 58–55; and the one from the Jordan Valley, 34–31.

As a rule the sum of the equivalents decreases in the direction of the lower layers, except in the fourth layer of Ben-Shemen and Djuania. The distribution of replaceable Ca, Mg, K, and Na in all these layers is very important. Ca, as we may conclude from the foregoing data, gradually decreases in all soils from the first layer to the fourth; Na, on the contrary, increases in the same direction. Thus the soils of Ben-Shemen form in a depth of more than 50 cm. a soil type similar to "Solonetz." According to its chemical properties Mg. resembles on one side the bivalent alkali earth metals and on the other side the alkali metals. Its properties have an influence also on its distribution among

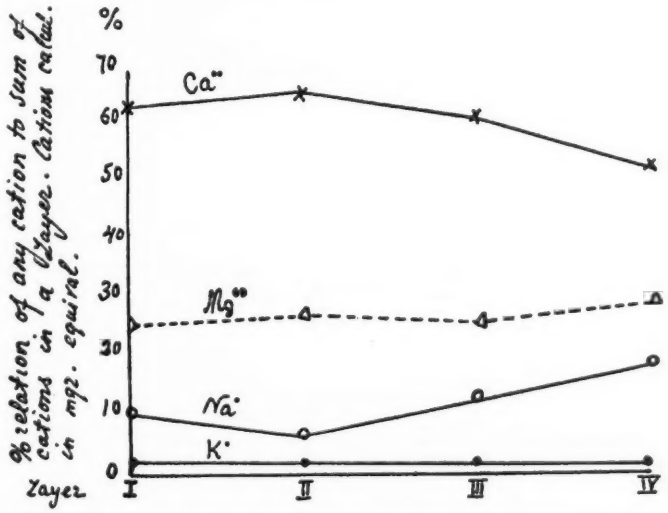


FIG. 1. DISTRIBUTION OF REPLACEABLE CATIONS IN BEN-SHEMEN SOIL

the layers of the soil. In the two samples (Ben-Shemen and Djuania) where replaceable Na is present in a large quantity, replaceable Mg is approximately constant, and only in the soil of the Jordan Valley does it increase in the lower layers. In this soil Mg seems to replace Na, which is present here in a small quantity.

Base exchange phenomena appear also in the natural conditions of the soil. The law of mass action, the quantity of any dissolved cation which exists in the soil in the form of any salt, and the replacing activity of the cation are the chief factors which regulate these phenomena. The quantity of the replaceable bases and their distribution among the layers of the soil can be observed as a result of an established equilibrium. The graphical interpretation of our figures of the replaceable bases in milligram-equivalents in the layers, illustrates

the existing relation between different replaceable cations. Figure 1 represents these relations for the Ben-Shemen soil. The shape of the curves for Ca and Na is remarkable. The state of saturation of the zeolites by Ca in each layer of the soil is inverse to the Na. This can be explained as a result of the mutual action between $CaH_2(CO_3)_2$ and NaCl in the soil solution and the zeolitic complex.

As a result of relatively much higher biochemical activity, Ca being influenced by the HCO_3^- and NO_3^- formed, dissolves much more completely in the solution of the upper layers of the soil. On the other hand NaCl, which is intensively leached out as a very soluble salt from the upper layers, accumu-

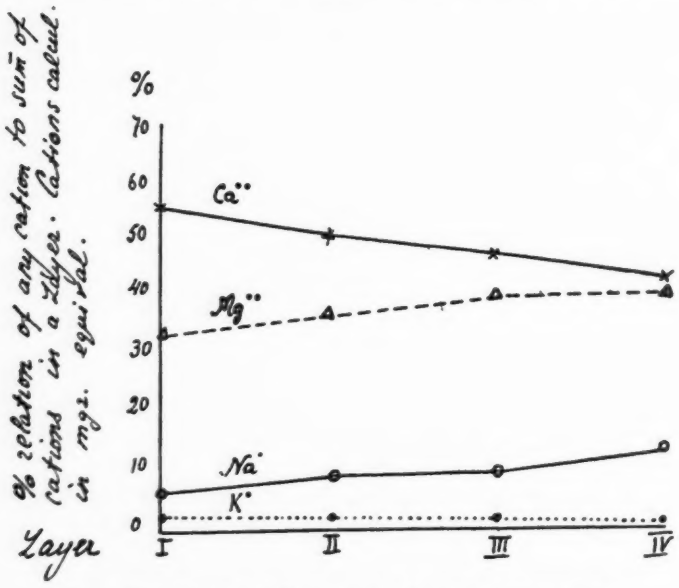


FIG. 2. DISTRIBUTION OF REPLACEABLE CATIONS IN DJUANIA SOIL

lates in the lower layers. This leads to a saturation of the upper layers by Ca. On the contrary, the increasing quantity of NaCl in the soil solution of the lower layers influences the enrichment of the zeolite by Na. A similar relation may be observed in the soil of Djuania (fig. 2) where the curves of Ca and Na are also inverse to each other.

In the soil of Ben-Shemen, Mg does not remain constant, but increases approximately in a parallel line to replaceable Na. Quite another relation can be observed between the replaceable cations in the soil of the Jordan Valley (Dagania). The inverse relation applies here to the curves of Ca and Mg (fig. 3) In the soil from Dagania, the easily dissolved salts are present in

very small quantities. In connection with this circumstance the colloid soil complex must be very slightly saturated by Na and K. The figures obtained for the replaceable Na and K in the investigated sample of the Dagania soil are very small when compared with the figures of Na and K found for Ben-Shemen and Djuania soils. The cation K is absolutely absent in the third and fourth layers. This is probably the reason why Mg appears as a cation replacing Ca in the lower layers. $MgCO_3$ is usually present in the calcareous soils together with $CaCO_3$ and the solubility of $MgCO_3$ is many times greater than that of $CaCO_3$. This is why $MgCO_3$, in the absence of any appreciable quantity of NaCl, appears in the lower layers as a salt displacing Ca and re-

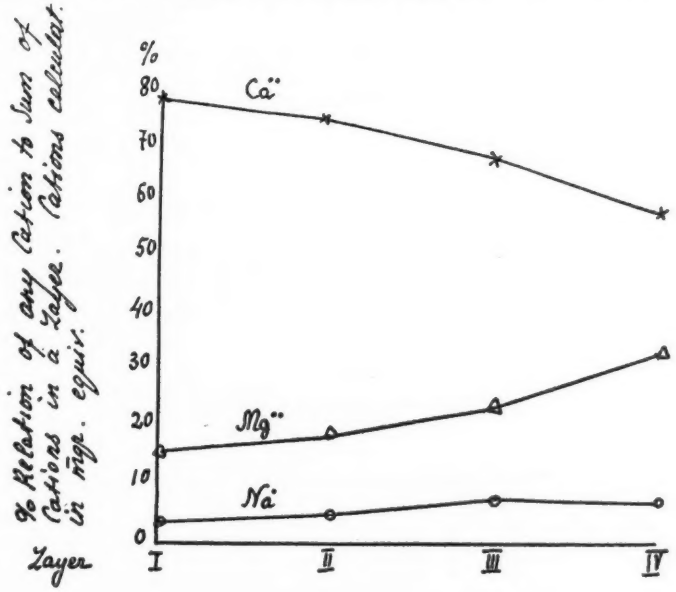


FIG. 3. DISTRIBUTION OF REPLACEABLE CATIONS IN DAGANIA SOIL

placing it in the colloid complex. When the soil possesses an important quantity of NaCl, Mg is usually replaced by Na.

The curves of the distribution of the replaceable bases which are present in the colloidal part of the soil in its different layers can be easily explained as a result of equilibrium between the soil and the cations taking part in the base exchange.

DISTRIBUTION OF CATIONS IN WATER EXTRACTS FROM DIFFERENT LAYERS OF SOIL

The supposition that the state of saturation of the colloidal complex of the soil is the result of the action of different cations which for a certain time are in

contact with the soil, leads the authors to admit the reverse phenomenon—the transition of the cations from the complex to the soil solution, i.e. if, for one reason or another, the solution will become poor in cations.

The decomposition pressure in the water medium of the soil zeolites saturated by different cations can not be the same. Apart from the properties of

TABLE 4

Water extracts from soil of Ben-Shemen

Ratio of soil to water 15:100

DEPTH	Ca	Mg	Na	NH_4	PO_4	SO_4	HCO_3	NO_3	Cl	SiO_2	TOTAL
cm.											
Amount of ions extracted from 100 gm. of dry soil, in mgm.											
0– 25	25.6	6.0	6.9	.....	Trace	.....	48.7	0.83	11.5	6.8	
25– 50	17.6	5.7	6.0	.....	.....	.....	35.0	0.52	11.7	5.5	
50– 75	13.4	5.2	19.1	.....	.....	.....	44.0	0.45	11.7	6.0	
75–100	15.0	6.8	30.1	.....	.....	.....	58.0	0.34	20.7	6.0	
Amount of ions extracted from 100 gm. of dry soil, in mgm. equivalent											
0– 25	1.28	0.50	0.30	.....	.....	.....	1.57	0.013	0.32	0.18	4.16
25– 50	0.88	0.47	0.26	.....	.....	.....	1.13	0.009	0.32	0.14	3.22
50– 75	0.67	0.42	0.83	.....	.....	.....	1.42	0.007	0.32	0.16	3.84
75–100	0.75	0.56	1.31	.....	.....	.....	1.87	0.005	0.58	0.16	5.24

TABLE 5

Water extracts from soil of Djuania

Ratio of soil to water 15:100

DEPTH	Ca	Mg	Na	NH_4	PO_4	SO_4	HCO_3	NO_3	Cl	SiO_2	TOTAL
cm.											
Amount of ions extracted from 100 gm. of dry soil, in mgm.											
0– 25	14.0	6.8	6.9	.....	Trace	.....	35.9	0.71	5.1	9.6	
25– 50	9.3	5.5	20.0	.....	.....	.....	42.5	0.39	4.1	10.5	
50– 75	6.6	3.2	29.9	.....	.....	.....	43.3	0.30	5.9	11.7	
75–100	6.1	2.8	43.2	.....	.....	.....	57.6	0.33	5.9	14.0	
Amount of ions extracted from 100 gm. of dry soil, in mgm. equivalents											
0– 25	0.70	0.56	0.30	.....	.....	.....	1.16	0.012	0.14	0.25	3.12
25– 50	0.46	0.45	0.87	.....	.....	.....	1.37	0.006	0.12	0.28	3.56
50– 75	0.33	0.26	1.30	.....	.....	.....	1.40	0.005	0.17	0.31	3.78
75–100	0.30	0.23	1.88	.....	.....	.....	1.86	0.005	0.17	0.37	4.82

the cations forming with the acid soil complex more or less stable compounds, the phenomena observed in the soil lead us to assume the presence of fractions of decreasing acidity which when saturated by the same cation may form com-

pounds of different stability. As a result of the kind of cation and of the saturation of the more or less acid "acidoides" of the soil the latter may contain a series of compounds of different composition and solidity which, when the soil is treated with water, forms a soil solution. The composition and the concentration of the cations in the solution reflect the state of saturation of the soil colloids by the bases. It is possible that a part of the cations of the relatively concentrated soil solution are formed by the hydrolysis of zeolitic compounds of the soil, but in order to obtain a more evident idea of the formation of the soil solution from the bases absorbed by the soil zeolites, the authors deemed it necessary to investigate more diluted water extracts of the soil, where naturally the hydrolysis occurs very intensively.

TABLE 6

Water extracts from soil of Dagania

Ratio of soil to water 15:100

DEPTH	Ca	Mg	Na	NH_4	PO_4	SO_4	HCO_3	NO_3	Cl	SiO_3	TOTAL
cm.											
Amount of ions extracted from 100 gm. of dry soil, in mgm.											
0– 25	14.5	4.5	3.5	Trace	.....	....	27.9	0.42	1.7	10.6	
25– 50	13.2	4.5	1.8	.....	.....	....	25.6	0.34	2.1	7.5	
50– 75	11.6	4.7	....	.....	.....	Trace	23.2	0.17	2.1	6.0	
75–100	11.2	5.8	....	.....	.....	3.2	23.2	0.22	2.3	6.0	
Amount of ions extracted from 100 gm. of dry soil, in mgm. equivalent											
0– 25	0.72	0.37	0.15	.....	.....	....	0.90	0.007	0.05	0.28	2.48
25– 50	0.65	0.37	0.08	.....	.....	....	0.83	0.006	0.06	0.20	2.20
50– 75	0.58	0.39	....	.....	.....	....	0.75	0.003	0.06	0.16	1.94
75–100	0.56	0.48	....	.....	.....	0.07	0.75	0.004	0.07	0.16	2.09

Extracts of 15 gm. of soil to 100 cc. of water were prepared for the following examinations. The results are represented in tables 4, 5, 6. The figures found for each cation expressing in per cent the total amount of cations for each layer of the soil of Ben-Shemen, Djuania and of Dagania are illustrated in figures of 4, 5, 6. When these curves are examined and compared with figures 1, 2, and 3 showing the distribution of the replaceable bases, some relations can be observed which bear the character of a law. We have observed before that the curves of the replaceable cations Ca and Na in the different layers of the Ben-Shemen soil are inverse to one another. The resembling draft of the curves for Ca and Na can be observed in the soil extract. The same relations can be seen by comparing the curves of the soil and the soil extract of the samples of Djuania and Dagania. *We have therefore some reason to believe that the distribution of cations of the water extract of the soils in a certain degree reflects the distribution of the same cations present as replaceable in the*

colloid complex of the soil. From the curves of figures 4, 5, and 6 further conclusions can be drawn. In the case of the soils highly saturated with Na this inverse character of the curves appears more evident than in the case of soils with a small quantity of absorbed Na. This phenomenon is caused by the character of the complex exposed to hydrolysis. As the observations show, the colloidal soil complex changes in relation to size and composition. The colloidal part of the soil which is the main bearer of the absorbing properties of the soil can be easily disintegrated and under certain conditions taken out from the soil both in a changed and an unchanged state. The water acting in a disintegrating and decomposing manner on the salt-like compounds of the

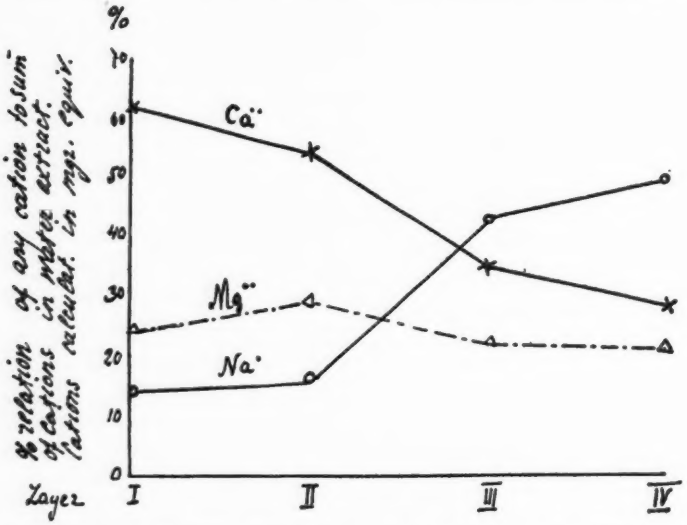


FIG. 4. DISTRIBUTION OF CATIONS IN WATER EXTRACTS FROM DIFFERENT LAYERS OF BEN SHEMEN SOIL

Ratio of Soil to Water 15:100

colloidal complex and on the split alumino-silicate nucleus is the cause of these phenomena. The parts of the complex highly saturated by Na are at first exposed to destruction because they belong to the less solid and easily hydrated, disintegrated, and hydrolyzed soil compounds. The soil complex exposed to intensive leaching not only loses its Na very rapidly but the soil groups which were bound with Na also submit to disintegration. The size of the colloidal absorbing complex generally decreases as a result of leaching and the parts more sensible to hydrolysis diminish too.

The soils of Dagania belong to the type of soils possessing a very low absorbing complex (S = 34 mgm. equivalent in 100 gm. of soil), and the presence

of only traces of dissolved salts demonstrates the strong leaching of these soil types. The remaining part of the soil absorbing complex is resistant to water and being also saturated with Ca and Mg gives very solid and slightly hydrolyzed compounds. For this reason the cation curves of the soil extract of Dagania are only slightly different from the curves of the replaceable cations in the same soil.

The colloidal soil complexes of Ben-Shemen and Djuania are in quite different conditions. The absorbing complex of these soils is very high ($S = 67$ and $S = 58$ mgm. equivalents in 100 gm. of soil) and the great part of it consists of

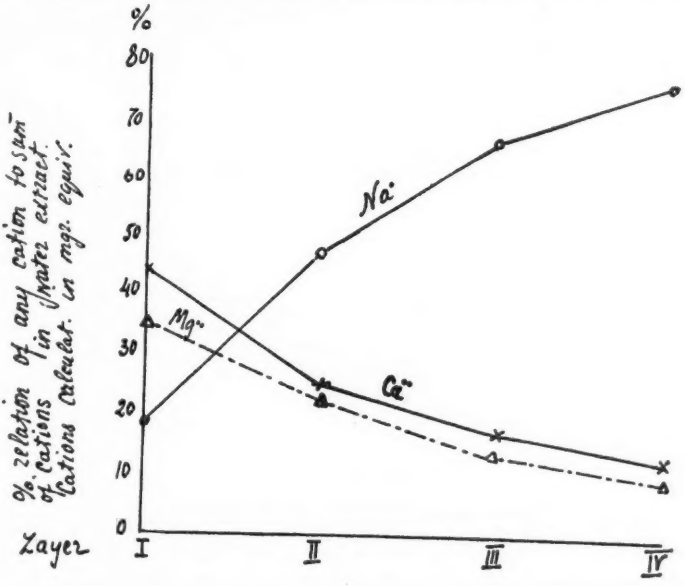


FIG. 5. DISTRIBUTION OF CATIONS IN WATER EXTRACTS FROM DIFFERENT LAYERS OF DJUANIA SOIL

Ratio of Soil to Water 15:100

unstable, easily hydrolyzed compounds. As a result of the water action, these soils are exposed to very strong hydrolysis and therefore the inverse character of the curves drawn for the cations of the water extract from the Djuania and Ben-Shemen soils is more conspicuous in comparison with the curves for the replaceable bases of the same soils.

Gedroiz (5) explains, by the properties of the replaceable cation, the degree of stability of the compound formed by the acid-absorbing part of the soil with the base. This property depends not only on the cation but also on the acid portion with which the cation is bound. It is very probable that there

exist in the soil (in this particular case in the mineral part of the soil) acid groups which form with the same cation some compounds of different stability. The saturation of the soil with a base takes place gradually. At first the very acid groups of the soil become saturated and form very solid compounds. By subsequent saturation with a base, less acid groups come into action and form with the bases less solid substances which readily decompose. If the soil contains some cations of different reactive power, then competition takes place at first for the formation of the compounds with the less acid groups where the cation is bound less strongly, and only later for the more solidly fixed cations.

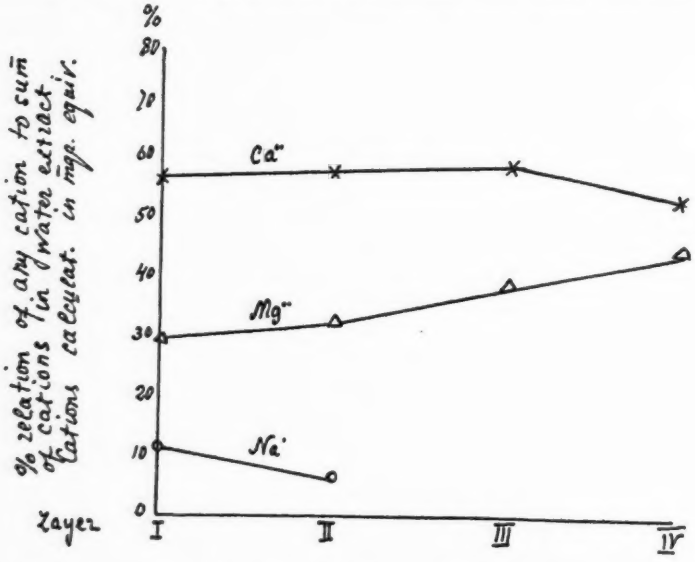


FIG. 6. DISTRIBUTION OF CATIONS IN WATER EXTRACTS FROM DIFFERENT LAYERS OF DAGANIA SOIL

Ratio of Soil to Water 15:100

In the case of hydrolysis, the first to decompose are the unstable compounds, formed with the cations during the highest state of saturation. This means, as we may expect, that the higher the amount of any replaceable cation in a colloidal complex the stronger the degree of hydrolysis.

Figure 7 shows the relation between the degree of hydrolysis and the amount of the cation in the absorbing complex of the soil. The amount in milligram-equivalents of a cation as a percentage of the total amount of replaceable bases in the soil sample is represented on the abscissa and the percentage of the quantity of milligram-equivalent of the same cation extracted from 100 gm. of soil to the total amount of replaceable bases in 100 gm. soil is represented on the

ordinate. The curves are plotted for Na and Ca for the soils of Djuania and Ben-Shemen. The curves show a high hydrolysis for Na in comparison to Ca, which agrees with the literature. According to Gedroiz (5) the replacing energy of different cations decreases in the following order: Ca, Mg, K, Na, the degree of hydrolysis of the replaceable bases being inverse for the same cations.

Another feature of these curves is the increasing degree of hydrolysis with an increase in the degree of saturation of the soil colloidal complex. This is more

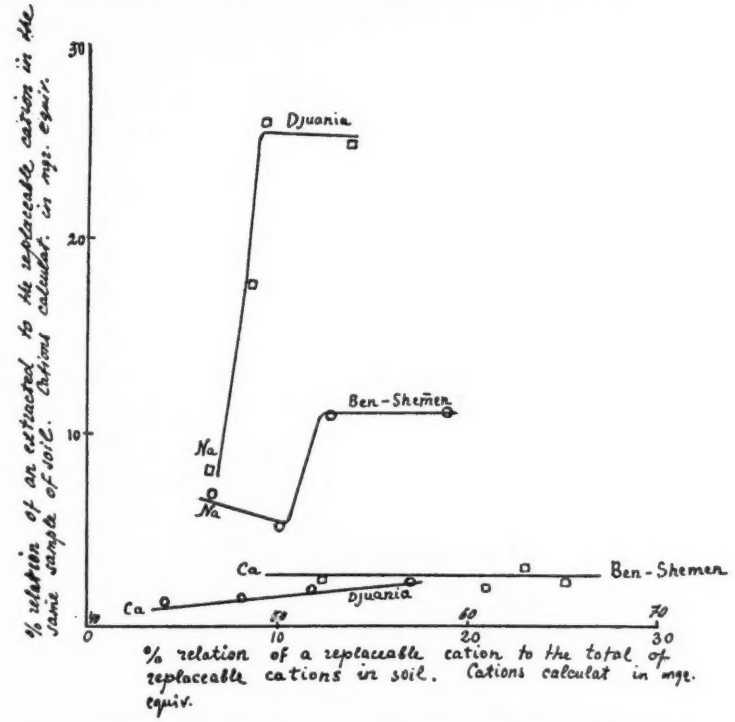


FIG. 7. HYDROLYSIS OF REPLACEABLE CA AND NA IN SOILS FROM BEN-SHEMEN AND DJUANIA

Ratio of Soil to Water 15:100

conspicuous for Na than for Ca. The results obtained support the theories of the author about the presence of some acid groups of different stability in the soil and of the formation of compounds of decreasing stability according to the increasing saturation of the colloidal soil complex by a cation.

INFLUENCE OF THE PROPORTION OF SOIL TO WATER ON THE COMPOSITION OF ANIONS AND CATIONS IN THE SOIL EXTRACT

In order to learn whether the aforementioned results for the soil extract can be applied to the much more concentrated soil extracts a series of water

TABLE 7

Amount of cations and anions in mgm. leached out from 100 gm. soil in different proportions of soil to water

Sample of Djuania soil. Calculated on dry matter

DEPTH	Ca				Mg				Na			
	Ratio of soil to water				Ratio of soil to water				Ratio of soil to water			
	100:100	50:100	25:100	15:100	100:100	50:100	25:100	15:100	100:100	50.100	25:100	15:100
cm.												
0– 25	4.0	7.3	11.6	14.0	1.9	2.9	5.0	6.8	1.8	3.7	6.4	6.9
25– 50	2.4	4.4	6.3	9.3	1.5	2.5	3.5	5.5	5.1	8.5	13.6	20.0
50– 75	...	2.6	4.7	6.6	...	1.7	2.3	3.2	...	12.0	18.9	29.9
75–100	...	2.1	3.9	6.1	...	1.5	1.8	2.8	...	16.1	25.8	43.2

DEPTH	HCO_3				Cl				SiO_3				NO_3			
	Ratio of soil to water				Ratio of soil to water				Ratio of soil to water				Ratio of soil to water			
	100:100	50:100	25:100	15:100	100:100	50:100	25:100	15:100	100:100	50:100	25:100	15:100	100:100	50:100	25:100	15:100
cm.																
0– 25	8.3	16.6	29.0	35.9	4.1	4.2	4.6	5.1	1.4	3.5	7.3	9.6	0.78	0.75	0.71	0.71
25– 50	10.3	18.6	27.7	42.5	2.9	3.0	3.5	4.1	1.6	4.1	7.2	10.5	0.34	0.36	0.40	0.39
50– 75	....	17.6	28.6	43.3	....	4.1	4.8	5.9	...	3.7	6.5	11.7	...	0.22	0.28	0.30
75–100	....	21.0	35.2	57.6	...	4.8	4.9	5.9	...	4.0	6.6	14.0	...	0.28	0.29	0.33

Amount of cations and anions in mgm. equivalents leached out from 100 gm. soil in different proportions of soil to water

DEPTH	Ca				Mg				Na				Total in mgm. equivalents			
	Ratio of soil to water				Ratio of soil to water				Ratio of soil to water				Ratio of soil to water			
	100:100	50:100	25:100	15:100	100:100	50:100	25:100	15:100	100:100	50:100	25:100	15:100	100:100	50:100	25:100	15:100
cms.																
0– 25	0.20	0.36	0.58	0.70	0.16	0.24	0.41	0.56	0.08	0.16	0.28	0.30	0.88	1.52	2.54	3.12
25– 50	0.12	0.22	0.31	0.46	0.12	0.21	0.29	0.45	0.22	0.37	0.59	0.87	0.92	1.60	2.38	3.56
50– 75	....	0.13	0.23	0.33	....	0.14	0.19	0.26	....	0.52	0.82	1.30	....	1.58	2.48	3.78
75–100	....	0.11	0.19	0.30	....	0.12	0.15	0.23	....	0.70	1.12	1.88	....	1.86	2.92	4.82

DEPTH	HCO_3				Cl				SiO_3				NO_3			
	Ratio of soil to water				Ratio of soil to water				Ratio of soil to water				Ratio of soil to water			
	100:100	50:100	25:100	15:100	100:100	50:100	25:100	15:100	100:100	50:100	25:100	15:100	100:100	50:100	25:100	15:100
cms.																
0– 25	0.27	0.54	0.94	1.16	0.12	0.12	0.13	0.14	0.04	0.09	0.19	0.25	0.013	0.012	0.012	0.012
25– 50	0.33	0.60	0.89	1.37	0.08	0.08	0.10	0.12	0.04	0.11	0.19	0.28	0.006	0.006	0.007	0.006
50– 75	....	0.57	0.92	1.40	....	0.12	0.14	0.17	....	0.10	0.17	0.31	....	0.004	0.005	0.005
75–100	....	0.68	1.14	1.86	....	0.14	0.14	0.17	....	0.10	0.17	0.37	....	0.005	0.005	0.005

Ions NH_4, SO_4, PO_4 absent.

extracts with an increasing proportion of soil to water were analyzed. Table 7 shows the numerical results of the analysis made with the water extracts of Djuania soil in the proportions 15, 25, 50, and 100 parts of soil to 100 parts of water. The figures in this table give valuable data for judging the origin of anions and cations of which the soil extract was formed. Some interesting properties may be observed in the anion part of extracts obtained from the investigated soil. First, it is remarkable that PO_4 and SO_4 are absent among the anions except in the fourth layer of Dagania soil (see tables 4, 5, and 6). Cl and NO_3 occupy a particular place between other anions. It is to be expected that salt present in soluble form in the soil will show by dilution with water that its concentration is inverse to the degree of dilution. The amount of milligram equivalents extracted from the same quantity of soil remains unchanged in all proportions of soil to water. From the numeral data concerning nitrates it may be observed that in all proportions of soil to water the quantity of NO_3 expressed in milligram-equivalents is unchangeable. From this it is evident that NO_3^- is a part of a salt present in a soluble form in the soil. As to Cl^- we can say that the salt of this anion is to some extent absorbed by the soil, although in its general properties it resembles the salt dissolved in water.

The two other anions of the soil extract—HCO_3^- and $SiO_3^=$—possess quite different properties. The quantity of both of these anions increases, in the extract from the same quantity of soil, with the increasing proportion of water to soil. In the extracts of our soils which are poor in organic matter we can not attribute the origin of HCO_3^- to CO_2. It would be more correct to suppose that simultaneously with the appearance of the bases in the solution after hydrolysis CO_2 is derived from the air and leads to the formation of HCO_3^- in the extract. The ion of $SiO_3^=$, the quantity of which also increases together with the increase of the proportion of water to soil, is evidently caused by the decomposition of some silicic soil compounds.

In soils rich in organic matter the methods of investigation of soil extracts render difficult any attempt to interpret the following question: "Does water in fact destroy the alumino-silicate nucleus of the primary particles of the colloidal complex or does it eject the fine powdered particles of the complex?" (7), even to make clear the question about the part played by the replaceable cations in these processes.

Our observations on the amount of $SiO_3^=$ in the soil extract obtained by treating the soil samples with water show as a general rule that the dissolved $SiO_3^=$ increases with the increase of the amount of water added to the soil and consequently there takes place a disintegration of the alumino-silicate particles. Should the opinions on the durability of the zeolitic nucleus dependent on replaceable bases prove to be accurate, we might expect the decomposition of the alumino-silicate nucleus in the lower layers of the soil where it is saturated with Na. This viewpoint would correspond to the well-known properties of replaceable Na, which intensively disintegrate the colloidal complex of the soil.

With regard to the relation which may probably exist between $SiO_3^=$ formed in a water extract and the replaceable Na the following facts can be observed in our data. In water extracts from the Ben-Shemen soils the amount of $SiO_3^=$ may be considered in all layers as a constant, without taking into account the unavoidable error of our method. In water extracts from the Djuania soil (table 7) where the proportions of the soil to water are 100, 50, and 25 to 100, one may assume the same amount of SiO_3 in all layers. An exception to the rule is in the two upper layers of the Dagania soil where we can observe an increase of $SiO_3^=$ in the water extracts.

On the basis of our data we can conclude from the continually increasing amount of replaceable Na in all investigated soils, that $SiO_3^=$ appears to be constant many times in the soil extracts and is only sometimes on the decrease. It follows that the appearance of this anion in water extracts is not dependent on the replaceable Na.

TABLE 8

Percentage of each cation in relation to total amount of cations extracted from 100 gm. soil

Taken for any layer in different proportions of soil to water

DEPTH	Ca				Mg				Na			
	Proportion of soil to water				Proportion of soil to water				Proportion of soil to water			
	100:100	50:100	25:100	15:100	100:100	50:100	25:100	15:100	100:100	50:100	25:100	15:100
cm.												
0– 25	45.5	47.3	45.7	44.9	36.3	31.6	32.3	35.9	18.2	21.1	22.0	19.2
25– 50	26.1	27.5	26.0	25.8	26.1	26.2	24.4	25.3	47.8	46.3	49.6	48.9
50– 75	....	16.5	18.6	17.5	....	17.7	15.3	14.3	....	65.8	66.1	68.2
75–100	....	11.8	13.0	12.5	....	12.9	10.3	9.5	....	75.3	76.7	78.0

It has been shown above in the samples of soils from Ben-Shemen, Djuania, and Dagania that the soil extract depends for its composition on the replaceable bases. The conclusions were based on the observations made on weak water extracts and it might be expected that with the increase of the concentration of the soil extract these relations would be different.

The following results were obtained by studying the water extract in various proportions of soil to water. By summing up the cations and anions of the soil extract we find that the total amount, expressed in milligram equivalents extracted from 100 gm. of soil, simultaneously increases with the proportion of water to soil, i.e. because of the influence of the water, a part of the insoluble compounds of the soil enters the solution. This process of transition of insoluble ingredients into the soil extract proves to be regular. If each cation is expressed in the percentage of the total amount of cations in the water extract in certain proportion of soil to water for any soil layer the percentage of each cation in the soil extract remains unchanged and independent of the general

increase of cations during the dilution. These calculations are reported in table 8 and the same relations are graphically interpreted in figure 8. *The distribution of the cations in a soil extract is independent of the degree of dilution*

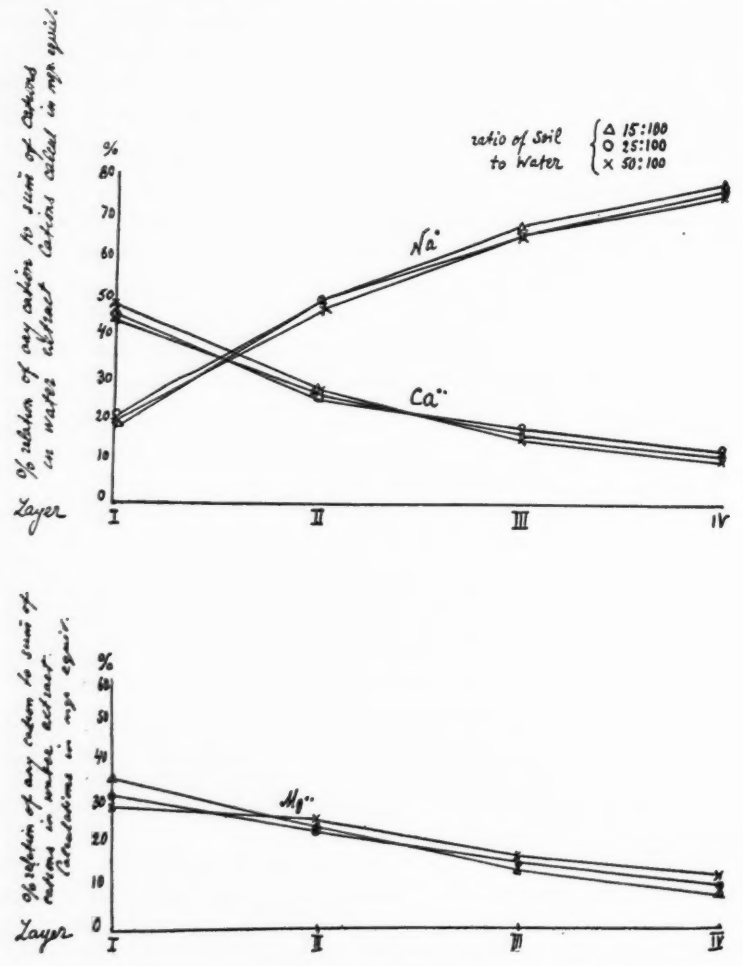


FIG. 8. THE INFLUENCE OF THE PROPORTION OF SOIL TO WATER ON THE DISTRIBUTION OF CATIONS IN DJUANIA SOIL EXTRACT

of soil in water and the appearance o each cation in solution strictly corresponds to the partial decomposition pressure of the compounds formed by replaceable cations and the zeolitic nucleus.

The results of our observations on water extracts of the soil lead to conclusions on the composition of the soil solution. It was found, as stated in the foregoing, that in water extracts of different proportions of soil to water the general amount of the extracted anions Cl^- and NO_3^- remains constant, but that HCO_3^-, on the contrary, increases with the increase of the proportion of water to soil. Hibbard (14) compared the composition of the soil solution obtained by the displacement method with the composition of the soil extract and drew the same conclusions in regard to the Cl, NO_3, and HCO_3 anions. We can thus establish the state of Cl^- and NO_3^- as parts of dissolved compounds in the fluid part of the soil but we may also decide on the grounds of the analysis of the soil extract about the origin of some other constituents of the soil solution. The constancy of the distribution of the cations in a soil extract both in low and high proportions of water to soil, permits us to draw conclusion also about the probability of the existence of this condition in the soil solution, i.e. in the relation of water to soil corresponding to the normal condition of soil moisture. It may be very probable that the soil solution of mineralized soils in its content of different cations and their distribution, reflects the state of saturation of the soil colloidal complex and its property of hydrolyzing under the influence of water.

SUMMARY

1. A study of the replaceable bases in three samples of soil from Ben-Shemen (Plain of Sharon), Djuania (Plain of Esdrealon), and Dagania (Valley of Jordan) demonstrated that the distribution of Ca and Na replaced from the first and second soil samples and of Ca and Mg from the third samples is inversed.

2. An examination of the water extracts of the soil (in the proportion of 15 gm. soil to 100 gm. water) of the aforementioned samples showed that the distribution curves of the corresponding cations in the extract according to the various soil layers conform to the distribution of replaceable bases of the soils. These curves, which are the results of the hydrolysis phenomena, are more distinctly expressed in the case of soils possessing large absorbing complexes.

3. An examination was made of a series of extracts from soils in which the proportion of soil to water was increased. It was found that, with the exception of one layer of the Dagania soil, the anions $PO_4^{\equiv}$ and $SO_4^{=}$ were absent in the soil extract. The amount of HCO_3^- and $SiO_3^=$ extracted from the soil was found to increase with dilution. Our observations show: (*a*) that the influence of the water leads to the decomposition of the alumino-silicate nucleus of the colloidal complex, and (*b*) that this decomposition does not depend on absorbed Na.

4. No_3^- and Cl^- bear the character of the anions of soluble salts. This corresponds to Hibbard's results.

5. The distribution of the cations in the soil extracts is not dependent on the degree of the increase of water in the soil. *The cations are distributed in the*

same percentage in all extracts, and the appearance of each cation in the extract corresponds to the partial decomposition pressure of the compounds formed by the absorbed cation with the alumino-silicate group.

6. Observations lead to the conclusion that in highly mineralized soils the composition of the soil solution relatively conforms with the composition of the replaceable bases in the soil colloidal complex, and to a certain degree reflects also the character of the same complex.

REFERENCES

(1) Bobko, E. W., and Askinasi, D. L. 1925 Bestimmung der Adsorptionkapasität und des Ungesättigkeitsgrades der Böden. *Ztschr. Pflanzenernähr. u. Düngung* 6: 99–127.

(2) Burd, J. S. 1925 Relation of biological processes to cation concentration in the soil. *Soil Sci.* 20: 269–283.

(3) Burd, J. S., and Martin, J. C. 1924 Secular and seasonal changes in the soil solution. *Soil Sci.* 18: 157–167.

(4) Gedroiz, K. K. 1923 The hydrochloric acid method for determining in the soil the cations present in adsorbed condition. *Soil Sci.* 16: 473.

(5) Gedroiz, K. K. 1927 Der adsorbierende Bodenkomplex, ed. 2. Nosowka Agr. Exp. Sta.

(6) Hibbard, P. 1923 Comparison of soil solution by displacement method and water extract. *Soil Sci.* 16: 165–171.

(7) Hissink, D. J. 1922 Beitrag zur Kenntnis der Adsorptionsvorgängim Boden. *Internatl. Mitt. Bodenk.* 12: 81–171.

(8) Hoagland, D. R. 1925 Physiological aspects of soil solution investigations. *Hilgardia* 1: 227–257.

(9) Shmuk, A., and Kurilo, M. 1926 *Jour. Landw. Wiss.* (*Moskau*) 3.

(10) Snell, F. D. 1921 Colorimetric Analysis. New York.

(11) Sobolew, F., and Dratschew, S. 1926 Ueber die Wirkung von Bearbeitung und Düngung auf die Dynamik der Bodenlösung und der absorbierten Kationen im Boden. *Jour. Landw. Wiss.* (*Moskau*) 2: 97.

(12) Stewart, G. R. 1918 Effect of season and crop growth in modifying the soil extract. *Jour. Agr. Res.* 12: 311–368.

(13) Whitney, M., and Cameron, F. K. 1903 The chemistry of the soil as related to crop production. U. S. Dept. Agr. Bur. Soils Bul. 22.

(14) Wiegner, G., Gallay, R., and Gessner, H. 1924 Wasserbindung im Boden. *Kolloid Ztschr.* 35: 313–322.

THE TOLERANCE LIMIT OF SEEDLINGS FOR ALUMINUM AND IRON AND THE ANTAGONISM OF CALCIUM

JOHN R. SKEEN

University of Pennsylvania

Received for publication September 21, 1928

That the presence of Al in the soil is a factor in the distribution of plants was first shown by Hartwell and Pember (7). The work of many other investigators has established the fact. Blair and Prince (3) show that the toxic effect of a soil may be due to the presence of Al and Fe while entirely independent of pH. Quoting from a later work of these authors (4) ". . . . enough work has been done to show that acids decrease pH and increase the amount of active Al in the soil."

That the only significant part of the soil with respect to plant nutrition is the soil solution, is well established. Wilting point determinations show that only a fraction of the capillary water is available for plant use; further, cell walls of roots and root hairs are many times thicker than the hygroscopic water film immediately surrounding the soil particles. In a complex colloid system as presented by soils, a prediction of the equilibrium established in the solution from total analysis data is extremely hazardous. Blair and Prince (3) demonstrated the benefit to be derived by "heavy application of acid phosphate to toxic soils" and Magistad (15) indicates the solubility of Al with respect to falling pH and the presence of phosphates.

From total phosphate determinations and pH of the soil, together with p.p.m. Fe and Al in the soil solution, the writer attempted to account for the quality of five soils obtained in Berks and Montgomery Counties in the summer of 1926. Three of the soils were unquestioned "bad producers" and two, used as checks, were said to be excellent. The pH's of the latter were 5.7 and 6.6 whereas those of the former were uniformly low, from 4.3 to 4.6. Determinations showed no significant differences in total phosphate of the five soils, whereas Fe and Al were present in from 0.1 to 1.5 p.p.m. in all cases. That the toxicity of free acid does not explain soil quality has been demonstrated in a previous paper (16) wherein the important rôle played by Ca has been suggested.

It is the object of this paper to establish the limits of Al and Fe concentration that can be tolerated by two type plants, *Lupinus albus* and *Phaseolus vulgaris nanus* and to demonstrate the antagonistic action of Ca for these toxic elements.

The method used is essentially that reported in a preceding paper (16). The growth of the primary radical in millimeters is reported at intervals of 24

hours and the solutions into which the seedlings were put were changed every day. Average temperatures were determined by means of a thermograph.

RESULTS

Table 1 shows the toxic effect of Fe and the antagonism of Ca for Phaseolus. Three separate experiments were performed but the results of only one are presented. The tendency was the same in all cases.

TABLE 1

Growth of radicals of Phaseolus with various treatments of Fe and Ca at an average temperature of 18°C.

SOLUTION		GROWTH DURING 24 HOURS	GROWTH DURING 48 HOURS	GROWTH DURING 72 HOURS	SIDE ROOTS
		mm.	mm.	mm.	
Water		7	8	8.2	Few and short
	p.p.m.				
Fe	0.05	8.3	9	9.5	Many blunt
	0.11	8	8.6	9.0	Many blunt
	0.23	7.7	7.7	7.0	Few blunt
	0.35	8.5	9.8	Dead	Few blunt
	p.p.m.				
Ca	5.0	13	22	28	Numerous, medium
+ Fe	0.05	16	28	38	Numerous, medium
+	0.11	13	20	23	Few, medium
+	0.23	13	22	29	Few, blunt
+	0.35	12	19	24	Few, blunt
	p.p.m.				
Ca	10.0	17	31	45	Numerous, long
+ Fe	0.05	16	31	43	Numerous, long
+	0.11	17	31	43	Numerous, long
+	0.23	18	35	51	Few, medium
+	0.35	20	43	63	Few short
	p.p.m.				
Ca	33.0	19	38	61	Numerous, long
+ Fe	0.05	18	39	69	Numerous, long
+	0.11	15	34	51	Numerous, long
+	0.23	13	27	42	Few, medium
+	0.35	16	35	54	Few, medium

The number and length of side roots are as important criteria with this type of plant as the length of the radical. It appears that small quantities of Fe accelerate growth with respect to distilled water but to no great extent when used alone. It is significant to note that the presence of 0.35 p.p.m. Fe is lethal to the radicals in three days although the tops continue apparently normal. Although side roots appear in solutions containing Fe, their growth is greatly inhibited.

There can be no question of the antagonistic effect of Ca for Fe in the concentrations used; there is not an exception to the contrary. That the toxic action of Fe is not completely antagonized is just as apparent.

It is seen that 0.05 p.p.m. Fe accelerates the growth of beans in the presence of 5.0 p.p.m. Ca. whereas increasing the concentration of Ca with the same amount of Fe produces a growth of radical and side roots as good as that in Ca alone. Within the time limit of the experiment, the toxic effect of 0.11

TABLE 2

Growth of radicals of Lupinus albus in various solutions of Fe and Ca at an average temperature of 17°C.

SOLUTION		GROWTH DURING 24 HOURS	GROWTH DURING 48 HOURS	GROWTH DURING 72 HOURS	GROWTH DURING 96 HOURS
		mm.	*mm.*	*mm.*	*mm.*
Water		14	21	25	28
	p.p.m.				
Fe	0.11	19	26	29	31
	0.23	17	20	27	28
	0.50	18	23	28	29
	0.70	17	20	24	24
	p.p.m.				
Ca	5.0	26	48	67	79
	+ Fe 0.11	30	49	59	66
	+ 0.23	29	42	54	58
	+ 0.50	25	40	52	56
	+ 0.70	25	48	62	69
	p.p.m.				
Ca	10.0	28	48	62	70
	+ Fe 0.11	26	38	52	65
	+ 0.23	22	40	49	51
	+ 0.50	16	35	52	63
	+ 0.70	24	50	63	68
	p.p.m.				
Ca	33.0	28	45	58	68
	+ Fe 0.11	28	47	62	82
	+ 0.23	27	43	52	61
	+ 0.50	23	40	52	64
	+ 0.70	29	56	76	82

p.p.m. Fe is entirely overcome by 10.0 p.p.m. Ca. On increasing the Fe concentration, slight ill effects are observed no matter how much Ca is added.

Growth acceleration occurs in 10 p.p.m. Ca with an Fe concentration of 0.23 and 0.35 p.p.m. but the number of side roots is less than in Ca alone. When 33.0 p.p.m. Ca is used with Fe, there is a slight decrease in growth of the radical with increased Fe concentration and also a noticeable decrease in the number of side roots although in no case were they observed to be blunted and abortive.

TABLE 3

Growth of radical of Lupinus albus at an average temperature of 23°C. in various solutions of Fe and Ca

SOLUTION		GROWTH DURING 24 HOURS	GROWTH DURING 48 HOURS	GROWTH DURING 72 HOURS	GROWTH DURING 96 HOURS	GROWTH DURING 120 HOURS	SIDE ROOTS
		mm.	*mm.*	*mm.*	*mm.*	*mm.*	
Water		18.5	24	26	27	27	
	p.p.m.						
Fe	0.2	18.5	28	31	32	32	Normal
	1.0	15	21	25	25	25	Blunt
	2.0	Dead	.....	.....	.....	.....	None
	p.p.m.						
Ca	5.0	22	44	52	55	53	
	+ Fe 0.2	24	42	50	55	56	Normal
	+ 1.0	22	40	52	58	60	Abortive, short
	+ 2.0	11	19.5	22	22.5	23	Blunt

TABLE 4

Growth of radicals of Lupinus albus at an average temperature of 29°C. in various solutions of Ca and Fe

SOLUTION		GROWTH DURING 24 HOURS	GROWTH DURING 48 HOURS	GROWTH DURING 72 HOURS	SIDE ROOTS AND RADICAL
		mm.	*mm.*	*mm.*	
Water		16	20	23	Loose turgidity (3 days)
	p.p.m.				
Fe	0.2	10.5	14	14	Loose turgidity, markedly (3 days)
	0.5	11.5	15	15	Loose turgidity (2 days), side roots blunt
	1.0	8	10	10	Loose turgidity (2 days), side roots blunt
	p.p.m.				
Ca	5.0	16	25.5	29	Side roots well developed
	+ Fe 0.2	17	27	29	Side roots normal
	+ 0.5	20	33	36.5	Side roots blunt
	+ 1.0	16	24	26	Side roots few and blunt
	p.p.m.				
Ca	10.0	17	30	35	Side roots well developed
	+ Fe 0.2	17	33	39	Side roots normal
	+ 0.5	20.5	33	39	Side roots blunt
	+ 1.0	22	30	32	Side roots few and blunt

TABLE 5

Growth of radicals of Lupinus albus when grown in various solutions of Al, Fe and Ca at an average temperature of 21°C.

SOLUTION	GROWTH DURING 1ST DAY	GROWTH DURING 2ND DAY	GROWTH DURING 3RD DAY	GROWTH DURING 4TH DAY	GROWTH DURING 5TH DAY	GROWTH DURING 6TH DAY	GROWTH DURING 7TH DAY	GROWTH DURING 8TH DAY	
	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	
Water	16	23	24	27	27.5	28	28	28	Loss of turgidity in 7 days
	p.p.m.								
Fe 0.5	17	20.5	24	26	27.5	27.0	27.0	27.0	Loss of turgidity in 8 days
1.0	12.3	18	19.7	21	21	21	20	20	Dead in 7 days
2.0	Dead							8	
	p.p.m.								
Ca 5.0	24	41	55	62	69	73	76	74	
+ Fe 0.5	21	39	49	58	62	62	63	63	
+ 1.0	19	35	43	49	53	56	57	57	
+ 2.0	12	19	23	26	28	29.5	29.5	29.5	Loss of turgidity in 8 days
	p.p.m.								
Ca 10.0	19	37	50	63	71	73	74	74	
+ Fe 0.5	20	44	57	67	71	73.5	73	73	
+ 1.0	20	37	43	48	51	52	52.5	53	
+ 2.0	13	23	29	32.5	35	37	39	39.5	
	p.p.m.								
Ca 20.0	19	41	56	67	73	77.5	78	78	
+ Fe 2.0	14	26	32	39	44	47	42	42	
Al 0.5	15	25	33	41	44	47	47	47	Radical distorted
1.0	15	29	37.5	41	42	43	43	42	Radical distorted
2.0	7	7.5	8	8	8	8	8	8	Turgid, dead (?) in 3 days
	p.p.m.								
Ca 5.0	24	41	55	62	69	73	76	74	
+ Al 0.5	18	32	45	57	66	76	81	82	
+ 1.0	13.5	29.5	40.5	55	66	75	78	79	Radical distorted
+ 2.0	11	19.5	27	31	31	31	31	30	Radical distorted
	p.p.m.								
Ca 10.0	19	37	50	63	71	73	74	74	
+ Al 0.5	16	35	49	62	74	79	83	84	
+ 1.0	18.5	34	44	52	54	55	54	54	Slight distortion
+ 2.0	10	19	24	27.5	28	28	28	28	Radical distorted
	p.p.m.								
Ca 20.0	19	41	56	67	73	77.5	78	78	
+ Al 2.0	13.5	28	40	43	43	43	44	44	Radical distorted

There are at hand five separate experiments on lupines performed from February 1–28, 1928 with Ca and Fe. The concentrations ranged from 0.05 to 2.31 p.p.m. Fe. Some results are given in table 2. In Fe concentrations of 0.05 to 0.5 p.p.m., in single salt solution, there is a rather decided acceleration in the growth rate of the radical for from one to two days depending on the Fe content of the solutions. After the first day, growth closely parallels that in distilled water and the ill effects of such Fe concentrations are no greater than those produced in distilled water. A concentration of approximately 1.15 p.p.m. Fe in single salt solution is required to kill lupines in three days. It is recalled that 0.35 p.p.m. Fe was lethal to Phaseolus in the same time limit. This difference in resistance to Fe parallels the contrasting response of these two plants to distilled water and Ca as before shown.

Table 2 shows again the beneficial action of Ca, but the response with lupines is not so marked as that with beans. (Or perhaps it is better to say that lupines are more resistant to the harmful action of distilled water than are beans.) The antagonism of Ca for Fe is again apparent. Fe used with Ca shows a growth acceleration, as compared to Ca alone, in two places, as was the case with Phaseolus. Concentrations of 0.11 and 0.23 p.p.m. Fe with 5 p.p.m. Ca cause an acceleration of growth as compared to 5 p.p.m. Ca alone for at least 24 hours, the rate being approximate to Ca thereafter. As little as 5.0 p.p.m. Ca seems completely to overcome tne toxicity of 0.5 and 0.7 p.p.m. Fe within the time limit of the experiments. The comparable Fe concentration with Phaseolus is approximately 0.35 p.p.m.

With 33.0 p.p.m. Ca + 0.7 p.p.m. Fe there seems to be a tendency for growth acceleration. Other combinations produce apparently normal radicals.

The data recorded in tables 3 and 5 are typical of the results obtained with lupines during June, 1928. The variation is only in degree. Growth acceleration occurs in 0.2 p.p.m. Fe for three days as compared with distilled water. But increase in growth with 0.5 p.p.m. Fe is noticeable for only 24 hours. Table 2 shows a comparable effect with 0.7 p.p.m. Fe. One p.p.m. Fe is lethal in 7 days and 2.0 p.p.m. Fe in 1 day. The beneficial effects of Ca again appear.

The experiments recorded in tables 3 and 4 were done within two weeks of each other and the only variable is temperature; materials, seeds, and method were exactly the same. At the higher temperature, the toxicity of Fe is more pronounced. A concentration of 0.2 p.p.m. is decidedly toxic, and loss of turgidity results in three days. Growth is distinctly less than that produced in distilled water at this concentration. In an Fe concentration of 1.0 p.p.m., a marked toxic action is observed. A comparison of the figures in tables 3 and 4, indicates that at 29°C., 0.2 p.p.m. Fe is more toxic than 1.0 p.p.m. Fe at 23°C. The influence of temperature is decided.

It is seen also that the response to Ca at the higher temperature contrasts with the reaction at lower temperatures. Lupines profit by increased additions of Ca in single salt solution at 29°C., in which respect they react as Phaseolus at 17–23°C.

At a temperature of 29°C., it is almost impossible to obtain uniform, dependable, and reproducible results with Phaseolus. Generally considered to be hardy plants, they are susceptible to a number of diseases which are particularly active at the higher temperatures. The reaction of Phaseolus at 29°C. would be interesting to know, but it is hardly possible to ascertain by this technique. The results obtained are, therefore, not presented.

Table 5 shows the results of an experiment on lupines as the test plant with the temperature at 21°C. The contrasted effects of Al and Fe are to be noted. Loss of turgidity is a rather convincing criteria of death. Certain concentrations of Fe cause the radical tip to become flabby within several days,

TABLE 6

Effect of various concentrations of Al and Ca on the growth of lupines at a temperature of 29°C.

SOLUTION		GROWTH DURING 24 HOURS	GROWTH DURING 48 HOURS	GROWTH DURING 72 HOURS	SIDE ROOTS AND RADICAL
Water		16	20	23	Loss of turgidity in 3 days
	p.p.m.				
Al	0.2	15	22	24	Side roots few and blunt
	0.5	15	24	26	Tips very scaly
	1.0	12	17.5	18	Cortex contracted 5 mm. from tip
	2.0	6.5	6.5	6.5	Apparently dead
	p.p.m.				
Ca	5.0	16	25	29	
	+ Al 0.2	16.5	33	40.5	Nearly normal
	+ 0.5	16	28	31	Distortion, side roots few and blunt
	+ 1.0	17	26	28	Distortion, side roots few and blunt
	+ 2.0	9	13	13	In 24 hours tip very brittle and scaly
	p.p.m.				
Ca	10.0	17	30	35	
	+ Al 0.2	18	34	41	Normal
	+ 0.5	15	24	29	Approach normality
	+ 1.0	14	18	19	Distorted, side roots blunt
	+ 2.0	12	18	20	In 24 hours tips scaly but not brittle

as already pointed out. But in no case with Al was the radical tip observed to be in the least altered in this regard. The ultimate toxic action differs greatly from that with Fe. Grown in Al solutions of 0.5 p.p.m. or more, the radicals bend in all directions and are greatly distorted, the distortion apparently increasing with increase in Al concentration. This phenomenon was not observed in the case of Fe. In single salt solutions of Al, the epidermis of the radicals was observed to become scaly and very brittle. The brittle character apparently continued into the cortex causing the radicals to break wherever they bent unless great care was taken. Fe was never observed to have this effect. The criteria of death with Al is not apparent to the writer.

The following peculiarities in growth are noted: Used in single salt solution, Al causes a great growth stimulation to the radical in comparison with distilled water. This contrasts sharply with Fe at the same concentrations (0.5 and 1.0 p.p.m.). With Ca, it is seen that 0.5 and 1.0 p.p.m. Al allow nearly normal growth of radicals after, perhaps, the first day. It would almost seem that the presence of Al were beneficial to lupines.

But with a concentration of 2.0 p.p.m. Al, a rather direct comparison of toxicity with Fe is obtained. Used alone or with 5.0, 10.0, or 20.0 p.p.m. Ca, the rate of growth compares with that in the various solutions of Fe. Used in sufficient concentration, Al is approximately as toxic as Fe.

Table 6 shows the effect of various concentrations of Al and Ca on the growth of lupines at a temperature of 29°C.

The growth depression with increase in temperature is again observed. Compared with distilled water, 1.0 p.p.m. Al is significantly toxic. At 21°C. an excellent growth was obtained at the same concentration. A parallel effect has been shown for Fe. The antagonistic action of Ca appears again although not so efficiently as at the lower temperature.

Comparing the results of table 6 with those of table 4 (temperature in both cases 29°C.), it is seen that Al depresses growth less than does Fe at the same concentrations and even when the initial growth depression with Al is of the same order as with Fe, elongation continues in the former solutions while ceasing in the latter. The results reported in table 5 are checked at the higher temperatures.

DISCUSSION

The response of lupines and Phaseolus to distilled water and Ca has been discussed in a previous paper (16). This paper concerns itself with the varied behavior of the plants to Fe and Al; to the antagonism of Ca for these elements; and the possible effect of temperature on toxic action.

1. At temperatures of from 17–21°C., in the same time interval, 0.35 p.p.m. Fe is toxic and lethal to beans while a concentration of 1.15 p.p.m. is lethal to lupines. It requires about three times the Fe concentration to produce the same result with lupines as with beans. This relative sensitivity of the two plants is seen throughout the work and parallels the sensitivity to distilled water.

Calculated to molar concentrations, there is much evidence (8, 10, 16) that the toxicity of heavy metals is greater than the toxicity of free acid. At a pH of 4.5, Phaseolus just about survives, that is to say at a molar HCl concentration of 0.00003. The same plant just survives with a molar Fe concentration of 0.000004. It would seem that the Fe ion is approximately seven times as efficient as the H-ion in its killing action.

The record for lupines will not permit such close comparison. They are known to survive for one day at a pH of 3.8 and for at least four days with a pH of 4.1. The limit of toleration is approximately 0.0001 *M* HCl. With Fe,

survival is at a molar concentration of 0.000018 (as chloride). With this plant, it again appears that the Fe ion is much more toxic than the H ion. Were the calculations in terms of normalities, the difference in toxicity would be multiplied by three, but the weight of evidence indicates that the effect is not due to valence in the case of heavy metals.

The writer offers no explanation for the different physiological toxicity of Fe and Al. That Al is lethal to plants cannot be denied, but why the root tips do not loose their turgidity is not apparent. In weak concentrations Al seems less toxic than Fe; stimulation of growth would suggest this. Work has been done to explain this action but no definite evidence has been obtained. The opinion is suggested that Fe is relatively immobile while Al is mobile. Kratzman (11) has shown the presence of Al in 130 plants species and finds no correlation of the relative presence or absence with plant families. His method indicates that Al is stored in plant cells in much the same manner as calcium oxalate crystals are found, and he suggests that "some plants may justly be called aluminum plants." Hoffer and Carr (9) have demonstrated the mobility of Al in corn. McHargue (14) and Hoffer and Carr (9) have demonstrated the presence of Fe in seeds; and its presence in chloroplasts can be demonstrated with little trouble at any time. This question seems to the writer to be indissolubly linked with permeability, not only of peripheral cells but of contiguous cells.

2. It is doubtful how far these data can be used to explain why one soil with low pH is toxic whereas another is not. That Ca is a factor is reasonable to suppose, but there are many others. However some evidence is at hand bearing on this point.

Six-inch pots were filled in duplicate with six soils [for analysis of four soils see previous paper (16)]. Five of these soils were known to lack plant-food and to be low in Ca; the fifth was regularly limed every year. Each pot was planted with 12 bean seedlings. One series was watered with distilled water, the second and third with 3.3 p.p.m. Fe. But to the third series had been added 5 gm. of $CaCO_3$ per pot. Approximately 14 liters of solution were used to each pot added at 15 intervals. Table 7 shows the tendency.

In all cases the presence of free Fe in the soil solution produced a stunted crop, and, with one exception, the addition of Ca to the soils almost entirely overcame the toxicity of the Fe. The use as a check on this point of a soil which had been limed for several years sustains the opinion that lime in the soil solution is an important ecologic factor even when the pH is abnormally low, and further, that lime in the soil solution may completely antagonize the toxic action of traces of Fe. The antagonism of Ca for Al has been shown.

From the data presented, it is doubtful if Phaseolus can survive an Fe or Al concentration exceeding 0.35 p.p.m. no matter how much Ca be present. With lupines the limit is placed at about 1 p.p.m. Fe or Al with the presence of a minimum concentration of Ca.

That Al is present in the soil at about this concentration, although not

demonstrated, is to be expected from the work of Magistad (15). Fe parallels the solubility of Al to a great extent. With the turning point of an Fe and Al solubility curve at pH 5.0, the two metals are brought into the soil solution in increasing concentration depending on the phosphate content of the soil. These metals are about five times as efficient in their toxic action as the H ion but their toxic action will depend on the Ca concentration of the soil solution.

TABLE 7

Weight of tops of Phaseolus sp. grown in pots with six different soils

One soil series is untreated; the second and third series show the effect of Fe and the antagonism of Ca.

SOIL	DISTILLED WATER ONLY			3.3 P.P.M. Fe (NO $CaCO_3$)			3.3 P.P.M. Fe, 5 GM. $CaCO_3$ PER POT		
	Number plants per pot	Average weight tops 1 plant	Nodules	Number plants per pot	Average weight tops 1 plant	Nodules	Number plants per pot	Average weight pots 1 plant	Nodules
		gm.			gm.			gm.	
1	9	3.5		10	3.2		11	3.5	
1	11	4.1		10	2.8		11	3.7	
Average.....		3.8	Few		3.0	None		3.6	Many
2	11	4.6		11	3.9		11	3.9	
2	11	4.1		11	3.6		10	3.4	
Average.....		4.35	Many, small		3.75	Few, small		3.65	Very many, large
3	11	3.5		10	2.2		10	3.2	
3	10	4.0		11	2.3		10	3.7	
Average.....		3.75	None		2.25	None		3.45	Many, large
4	11	3.4		10	2.8		10	3.9	
4	11	3.5		11	3.3		11	4.0	
Average.....		3.45	None		3.05	None		3.95	Many, large
Limed soil	11	4.2	Many	10	4.2	Many, small	11	4.2	Very many, large
5	8	3.7	None	10	2.8	None	9	3.9	Many, small

From Magistad's curve and from the data here presented, it is quite possible that a soil with a pH of 4.3 will show no evidence of toxicity should the phosphate and Ca content be high. Blair and Prince (3) and Burgess (5) have indicated that with lettuce, onions, and soybeans, the toxicity of soils is not due to pH but most probably to Al.

3. Leitch (13), Lehenbauer (12), Balls (1), and others have shown that the optimum temperature for the growth of plants is 29°C. From 2–29°C.,

growth and temperature can be expressed in a uniform curve; at higher temperatures the growth curve is very erratic, and no single law governs the tendency.

Behring (2) showed that the action of disinfectants is increased by a rise in temperature but the writer knows of no other work bearing on the increased toxicity of metals with rise in temperature.

The data presented show the increased toxicity of water at 29°C., the greater toxic action of Al and Fe at this temperature, and the marked beneficial effects of increased concentrations of Ca. The resistant lupine acts at 29°C. much the same as the relatively sensitive bean at 17–21°C. These data check the demonstrations of Hansteen-Cranner (6) but a discussion is reserved until more evidence is obtained.

It is the opinion of the writer, from the evidence presented, that the value of pH determinations of the soil is only in the prediction of the elements one may expect to find in the soil solution; that plants do not show a preference for a specific pH in the concentrations in which the H ion is usually found in the soil (16) but that plants may more correctly be called "calciphils" and "calciphobes" or, from Kratzman's investigations, "aluminaphils" and "aluminaphobes."

SUMMARY

With the condition and growth of the radicals of *Lupinus albus* and *Phaseolus vulgaris nanus* as criteria, the relative toxicity of Fe and Al to these plants and to each other was studied.

Some data are presented showing the increased toxicity of Al and Fe at 29°C.

L. albus is about three times as resistant to Al and Fe as is *Phaseolus*.

The Fe ion is about five to seven times as toxic as the H ion.

The different physiological toxicities of the Al and Fe ions are designated.

The antagonism of Ca for Al and Fe is demonstrated and from these data the limits of Al and Fe concentration that may exist in the soil solution are approximated for *L. albus* and for Phaseolus. Pot experiments are used to check water culture conclusions.

Experiments and citations are discussed in support of the opinion that cH[1] of itself is of little importance as an ecologic factor.

REFERENCES

(1) Balls, L. W. 1908 Temperature and Growth. *Ann. Bot. (London)* 22: 557–591.

(2) Behring, 1890 Ueber Desinfection, Desinfectionsmittel, and Desinfectionsmethoden. *Ztschr. Hyg. u.* Infektions krank. 9: 395–478.

(3) Blair, A. W., and Prince, A. L. 1923 Studies on the toxic properties of soils. *Soil Sci.* 15: 109–129.

[1] cH is the symbol for H-ion concentration. $pH = \log \frac{1}{cH}$.

(4) BLAIR, A. W., AND PRINCE, A. L. 1927 The relation of soil reaction to active aluminum. *Soil Sci.* 24: 205–213.

(5) BURGESS, P. S. 1923 A method for the determination of active aluminum in acid soils. *Soil Sci.* 15: 131–135.

(6) HANSTEEN-CRANNER, B. 1919 Beitrage Zur Biochemie und Physiologie der Zellwand und der plasmatischen Grenzschichten. *Ber. Deut. Bot. Gesell.* 37: 380–391.

(7) HARTWELL, B. L. AND PEMBER, F. R. 1918 The presence of aluminum as a reason for the difference in the effect of so-called acid soil on barley and rye. *Soil Sci.* 6: 259–279.

(8) HEALD, F. D. 1896 On the toxic effect of dilute solutions of acids and bases upon plants. *Bot. Gaz.* 22: 125–153.

(9) HOFFER, G. N., AND CARR, R. H. 1923 Accumulation of aluminum and iron compounds in corn plants and its probable relation to root rots. *Jour. Agr. Res.* 23: 801–824.

(10) KAHLENBERG, L., AND TRUE, R. H. 1896 On the toxic action of dissolved salts and their electrolytic dissociation. *Bot. Gaz.* 22: 81–124.

(11) KRATZMAN, E. 1913 Der mikrochemische Nachweis und die Verlveitung des Aluminiums in Pflanzenreich. *Sitzber. K. Akad. Wiss. (Vienna), Math. Naturw. Kl.* 122: 311–336.

(12) LEHENBAUER, P. A. 1914 Growth of maize seedlings in relation to temperature. Abs. in *Physiol. Res.* 1: 247–288.

(13) LEITCH, T. 1916 Some experiments on the influence of temperature on the rate of growth of Pisum sativum. *Ann. Bot. (London)* 30: 25–46.

(14) MCHARGUE, J. S. 1923 Iron and manganese content of certain species of seeds. *Jour. Agr. Res.* 23: 395–399.

(15) MAGISTAD, O. C. 1925 The aluminum content of the soil solution and its relation to soil reaction and plant growth. *Soil Sci.* 20: 181–226.

(16) SKEEN, J. R. 1929 Some reactions of seedlings to weak concentrations of HCl and Ca. *Soil Sci.* 26: 471–478.

(17) WHERRY, E. T. 1927 The soil reaction preferences of certain plant orders. *Wash. Acad. Sci. Jour.* 17: 148–149.

BOOK REVIEW

Lehrbuch der Agrikulturchemie (*Textbook of Agricultural Chemistry*). By E. HASELHOFF and E. BLANCK. Gebrüder Borntraeger, Berlin. Teil I. *Pflanzenernährungslehre* (*Plant Nutrition*). By E. BLANCK. 1927. Pp. 207, price, 10.50 M. Teil II. *Düngemittellehre* (*Fertilizers*). By E. HASELHOFF. 1928. Pp. 216, price, 12.0 M. Teil III. *Bodenlehre* (*Soils*). By E. BLANCK. 1928. Pp. 208, price, 11.40 M.

The purpose of these books, which are designed primarily for the agricultural student and the practical agriculturist, is to give a summary of the present status of investigation in the field of agricultural chemistry. Without going into a detailed review of the various individual investigations, the authors have presented a complete survey of the science of agricultural chemistry and its influence upon practical agriculture. No attempt has been made to review the literature completely or thoroughly; the authors have limited themselves to a discussion of the fundamental principles underlying the subject.

The first part, prepared by Prof. Blanck of the University of Göttingen, treats of the principles of plant nutrition and plant metabolism, including sections on the chemical composition of the organic and inorganic plant constituents and on the formation and transformation of organic matter in the plants. The second part, prepared by Prof. Haselhoff, deals with principles of fertilization and with natural and artificial fertilizers. The third part, prepared by Prof. Blanck, treats of soil science in its relation to geology, processes of weathering, the rôle of minerals and rocks in soil formation, influence of climate upon soil formation, chemical composition of soil, biological condition of soil, etc. The fourth part of this book dealing with animal feeding, by E. Haselhoff, is still to be published.

Each part is complete in itself and has detailed author and subject indexes. The treatment of the subject is authoritative throughout and the book should find its place in every agricultural library.

S. A. WAKSMAN.